



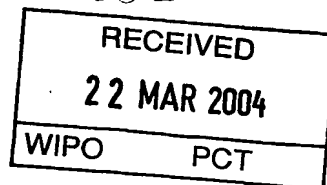
Europäisches
Patentamt

European
Patent Office

10/342800
04. März 2004
PCT/00 4 / 00 0 4 2 3

Office européen
des brevets

23 JUL



Bescheinigung

Certificate

Attestation

Die angehefteten Unterla-
gen stimmen mit der
ursprünglich eingereichten
Fassung der auf dem näch-
sten Blatt bezeichneten
europäischen Patentanmel-
dung überein.

The attached documents
are exact copies of the
European patent application
described on the following
page, as originally filed.

Les documents fixés à
cette attestation sont
conformes à la version
initialement déposée de
la demande de brevet
européen spécifiée à la
page suivante.

Patentanmeldung Nr. Patent application No. Demande de brevet n°

03026613.4

**PRIORITY
DOCUMENT**
SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17.1(a) OR (b)

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

BEST AVAILABLE COPY



Anmeldung Nr:
Application no.: 03026613.4
Demande no:

Anmeldetag:
Date of filing: 19.11.03
Date de dépôt:

Anmelder/Applicant(s)/Demandeur(s):

Max-Planck-Gesellschaft zur Förderung
der Wissenschaften e.V.
Hofgartenstrasse 8
80539 München
ALLEMAGNE

Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se référer à la description.)

L-amino acid oxidase with cytotoxic activity from Aplysia punctata

In Anspruch genommene Priorität(en) / Priority(ies) claimed /Priorité(s)
revendiquée(s)

Staat/Tag/Aktenzeichen/State/Date/File no./Pays/Date/Numéro de dépôt:

EP/20.01.03/EP 03001232

Internationale Patentklassifikation/International Patent Classification/
Classification internationale des brevets:

C12N9/00

Am Anmeldetag benannte Vertragsstaaten/Contracting states designated at date of
filing/Etats contractants désignées lors du dépôt:

AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LU MC NL
PT RO SE SI SK TR LI

WEICKMANN & WEICKMANN

Patentanwälte
European Patent Attorneys · European Trademark Attorneys

EPO - Munich
53

18. Nov. 2003

DIPL.-ING. H. WEICKMANN (bis 31.1.01)
DIPL.-ING. F. A. WEICKMANN
DIPL.-CHEM. B. HUBER
DR.-ING. H. LISKA
DIPL.-PHYS. DR. J. PRECHTEL
DIPL.-CHEM. DR. B. BOHM
DIPL.-CHEM. DR. W. WEISS
DIPL.-PHYS. DR. J. TIESMEYER
DIPL.-PHYS. DR. M. HERZOG
DIPL.-PHYS. B. RUTTENSBERGER
DIPL.-PHYS. DR.-ING. V. JORDAN
DIPL.-CHEM. DR. M. DEY
DIPL.-FORSTW. DR. J. LACHNIT

Our Reference:
29644P EP-1/WWDikt

Applicant:
Max-Planck-Gesellschaft zur
Förderung der Wissenschaften e.V.
Hofgartenstraße 8

80539 München

L-amino acid oxidase with cytotoxic activity from *Aplysia punctata*

L-amino acid oxidase with cytotoxic activity from *Aplysia punctata***Description**

5

The present invention relates to a cytotoxic polypeptide which is an L-amino acid oxidase isolated from the ink of the sea hare *Aplysia punctata*.

- 10 The sea hare *Aplysia* produces a pink-coloured ink, which has cytotoxic activity towards several eukaryotic cell lines. WO97/16457 discloses a partial sequence from an *Aplysia* protein, which allegedly has anti-tumor activity. Cyplasin L (558 aa, NCBI accession number 11967690) and cyplasin S (421 aa, 11967688; Petzelt and Werner, 2001, Cell Biology
- 15 International, 25(2):A23) both include parts of sequences disclosed in WO 97/16457. Cyplasin S exhibits 95% sequence identity to cyplasin L. Cyplasin L is produced in the nidamental gland but neither in the ink gland (including the mantle region) nor in the opaline gland of *Aplysia punctata*. Thus, it is concluded that cyplasin is not a component of *Aplysia* ink and is
- 20 not responsible for the cytotoxic activity of the *Aplysia* ink. A detailed description of *Aplysia* anatomy and a dissection guide can be found in the internet in Richard Fox, Invertebrate anatomy (1994, <http://www.science.lander.edu/rsfox/>).
- 25 The overall aim in tumor therapy is the selective eradication of transformed cells without harming healthy cells. Several glycoproteins isolated from sea hares (*Aplysia* species) have attracted attention because of their anti-tumor activity, e.g. aplysianin A from *Aplysia kurodai*, or cyplasins. The underlying mechanism for such activity has however not been elucidated
- 30 so far. Recombinant intracellular cyplasins seem to be non-toxic, whereas the extracellular cyplasin is cytotoxic (Petzelt et al., Neoplasia, 4:49-59, 2002).

WO 03/057726 discloses a cyplasin which is devoid of a functional secretory signal sequence. Since cyplasin only causes eukaryotic cell death from outside, the cyplasin of WO 03/057726 can thus be functionally expressed in eukaryotic cells without killing these cells. When acting from outside, cyplasin induced cell death is accompanied by fast depolymerization of the actin filaments. Expression of bioactive cyplasin S and L in prokaryotic host cells is not possible.

WO 02/31144 discloses a further cytotoxic factor isolated from the ink of *Aplysia punctata*. Fragments of the amino acid sequence of the factor are disclosed. No data were presented demonstrating that this factor has any oxidase function or has any properties related to an oxidase.

At least two main phenotypes of cell death are described: apoptosis, a genetically fixed physiological form of cell death, is accompanied by shrinkage, membrane blebbing, nuclear fragmentation, and final disintegration into so-called apoptotic bodies. In contrast, necrosis is a pathological process characterized by membrane disruption and cell swelling. Cell death induced by reactive oxygen and nitrogen species (ROS/NOS) might lead to apoptosis and necrosis but also to other forms of cell death, which cannot be clearly assigned to one of these main forms of cell death.

The cytotoxic factors derived from the sea hares so far have several disadvantages which might hamper its application. The biological function and the nature of the cytotoxic activity, which are prerequisites for the development of a lead compound, are not known so far. Aplysianin A contains a dinucleotide binding fold and the so-called "GG motif" which are found in many flavoproteins. The GG motif has also been described in cyplasins (Petzelt et al., supra). Based on this knowledge, the factors can be applied in its entirety only, because the domains relevant for proper function and cellular receptors are unknown. The administration of an

entire non-self protein to an animal or a human might cause severe immunologic complications.

The dinucleotide binding fold and the GG motif are found e.g. within the
5 N-terminal domain of FAD containing enzymes (e.g. reductases, dehydrogenases, hydroxylases, peroxidases, and oxidases). FAD containing enzymes can be classified into five groups GR1, GR2, FR, PCMH, and PO according to the sequences of their FAD binding domains and additional conserved sequence motifs (Dym and Eisenberg, Protein
10 Science, 10:1712-1728; 2001). The consensus sequence of GR1 and GR2 is GxGxxG. The GG motif RhGGRhxxT/S is commonly found in oxidases, e.g. L-amino acid oxidases, monoamino oxidases, polyamine oxidases, and putrescine oxidases, wherein x describes any amino acid, and h describes a hydrophobic amino acid.

15 L-amino acid oxidases catalyse the formation of H_2O_2 , ammonia, and an alpha keto acid from an amino acid in the presence of oxygen and H_2O (Geyer et al, 2001, Eur. J. Biochem. 268, 4044-4053). An L-lysine alpha oxidase (EC 1.4.3.14) for instance can be obtained from the fungus
20 Trichoderma spec. (Kusakabe et al., J. Biol. Chem. 10:976-981, 1980) which shows antimetastatic effects (Umanskii et al., Biull Eksp Biol Med. 109:458-9, 1990, Khaduev et al., Biull Eksp Biol Med. 112:419-22, 1991). The Trichoderma L-lysine oxidase is a dimer with a molecular weight of 112-119 kDa. A further L-lysine oxidase obtained from the fish Chub
25 mackerel is a dimer and has a molecular weight of 135 kDa (Jung et al., J. Immunol. 165:1491-1497, 2000) and induces apoptosis. Apoxin is an L-leucin oxidase from the rattlesnake (Crotalus atrox) venom which induces apoptosis in tumor cells and vascular endothelial cells in vitro (Torii et al., J. Biol. Chem. 272:9539-9542, 1997). A cytotoxic L-lysine alpha oxidase
30 is described in the art which penetrates into Jurkat cells and there activates oxidative deamination of L-lysine and correspondingly the peroxide formation. Conjugates of the enzyme with monoclonal antibodies

against the CD5 receptor cannot penetrate into the cells and are assumed to produce toxic H_2O_2 outside the cells. The conjugates have a reduced cytotoxic effect, although the effect of conjugation upon enzymatic activity is negligible (Zhukova et al., Vopr Med Khim 2001, 47:588-592). Another
5 L-lysine oxidase obtained from the snail *Achatina fulica* and producing H_2O_2 is found to have an antimicrobial effect. This oxidase might be useful as an agent against pathogenic bacteria (Ehare et al., 2002, FEBS Letters, 531:509-512).

10 Most known alpha amino acid oxidases which produce H_2O_2 possess a broad substrate specificity. The L-lysine alpha oxidase from *Trichoderma viride* (EC 1.4.3.14, Kusakabe et al., supra) is specific for lysine, but also oxidizes L-ornithine, L-phenylalanine, L-tyrosine, L-arginine, and L-histidine to a lesser extent. The L-lysine oxidase of Chub mackerel (EMBL,
15 AJ400781; Jung et al., supra) is specific for lysine and in addition transforms arginine, histidin, leucine, methionine, phenylalanine, and ornithine (specifity 40 fold reduced). Even if these enzymes could be cytotoxic due to their ability to produce H_2O_2 , a therapeutic use is hampered because substrates of these enzymes are available in the body
20 fluid in amounts sufficient to release H_2O_2 everywhere in the body. Under these conditions, possible negative side effects of H_2O_2 are difficult to eliminate.

25 In addition to H_2O_2 producing enzymes, cells possess a detoxification system which eliminates reactive oxygen species (ROS), in particular H_2O_2 . An important class of detoxifying peroxidases are peroxiredoxins. Peroxiredoxins comprise a class of highly conserved oxidases. In mammals, six different isoforms are known which catalyze the reduction of peroxides by using reducing equivalents that are provided by thioredoxin or
30 glutathione. During catalysis, peroxiredoxin I (Prx I) is inactivated by oxidation of the active site cysteine to cysteine sulfinic acid, a modification which is reversible upon removal of H_2O_2 . Previously, overexpression of

both Prx I and Prx II has been shown to render cells resistant to H_2O_2 induced apoptosis.

The problem underlying the present invention is the provision of a means
5 for selective generation of H_2O_2 in target tissues, e.g. in tumor tissues with
less toxic side effects upon normal cells. The solution is a cytotoxic
polypeptide which can be isolated from the ink of the sea hare *Aplysia*
punctata and which is a specific L-lysine and/or L-arginine oxidase
producing H_2O_2 or a fragment or derivative of said polypeptide. The activity
10 of the enzyme can be modulated by administration of substrate. The
enzyme provides a lead structure, and it can be used for target
identification.

A first aspect of the present invention is a purified polypeptide which
15 exhibits cytotoxic activity on tumor cells and which comprises the amino
acid sequence shown in SEQ ID NO: 2, 4, or 6, or a cytotoxic fragment
thereof. These sequences are derived from a cytotoxic 60 kDa protein
purified from crude ink of *Aplysia punctata* via anion exchange
chromatography and gel filtration (see examples 1 and 4). Thus, the
20 polypeptide or the fragment is termed APIT (*Aplysia punctata* ink toxin).
The purity of the fractions can be determined by SDS-PAGE and silver
staining.

The cytotoxic activity of APIT or the diluted crude ink can be measured by
25 the reduction of the metabolic activity of eukaryotic cells. A person skilled
in the art knows suitable methods and cell lines. For example, the
metabolic activity of Jurkat T cells can be measured by the addition of
WST-1, which is a tetrazolium salt converted by cellular enzymes of viable
cells, e.g. by the mitochondrial dehydrogenase, to a dark red formazan.
30 Therefore, the amount of formazan correlates with cell vitality. Formazan
can be determined photometrically at 450 nm. Further, dead eukaryotic
cells killed by APIT or the diluted crude ink can be counted by adding

propidium iodide (PI) at 1 μ g/ml in PBS and subsequent flow cytometer analysis. PI is a DNA binding dye which is taken up by dead cells with permeable membranes.

- 5 The cytotoxic activity of APIT is reduced by at least 70% after 10 min incubation at 60°C. At 70°C, the activity is almost absent, whereas 0°C to 50°C have no effect upon the activity. APIT shows a loss of activity with decrease of pH, with complete inactivation after 10 min pre-incubation at pH 3. After 30 min treatment with 6 M urea, the activity
10 of APIT is almost unaffected. At 8M urea, the activity is reduced by about 50% (example 3).

Tumor cells treated with APIT displays a morphology which is neither typical for apoptosis nor for necrosis but rather is typical for oxidative
15 damage induced cell death. Shrunken nuclei and lack of cell swelling are apoptotic, and early membrane permeabilization is a necrotic characteristic (example 2). The phenotype induced by APIT could be reproduced in Jurkat cells by treatment of the cells with concentrations of H_2O_2 > 200 μ M, indicating that H_2O_2 is the active compound in APIT cytotoxic effect.
20 H_2O_2 concentrations < 100 μ M induced apoptosis in Jurkat cells. In contrast to the mode of action of cyplasins, a depolymerization of the active filaments cannot be observed in APIT induced cell death, indicating that the mechanism of APIT action is distinct from that of cyplasins (Example 12).

25

By depriving possible substrates which can be converted into H_2O_2 from the culture medium of the tumor cells, it can be demonstrated that no further toxic effect of APIT upon tumor cells is present. Deprivation of L-lysine and L-arginine from the medium prevents cell death completely. In
30 a detailed analysis of the enzymatic activity of APIT, media containing single amino acids (20 L-amino acids, D-lysine) confirmed that L-lysine and/or L-arginine is converted into H_2O_2 and the respective alpha keto acid

to the same extent, whereas no conversion could be measured with any other of the remaining 18 L-amino acids and D-lysine (example 7). The production of H_2O_2 is independent of the presence of cells, however, the presence of cells reduces the amount of free H_2O_2 , which might be due to
5 detoxification of the medium by the cells. Catalase (a H_2O_2 hydrolyzing enzyme) prevents tumor cell death induced by purified APIT and by crude ink as well, confirming the conclusion that H_2O_2 is responsible for the ink mediated killing of tumor cells (example 6).

10 In summary, the data demonstrate that the polypeptide of SEQ ID NO: 2, 4, or 6 (APIT) is an oxidase which is capable to produce H_2O_2 . Particularly, the polypeptide is an alpha amino acid oxidase. More particularly, the polypeptide specifically converts L-lysine and/or L-arginine in the presence of O_2 and H_2O into an alpha keto acid, ammonia, and H_2O_2 . Thus, the
15 polypeptide is preferably an L-lysine and/or L-arginine oxidase.

A characteristic feature of the active fractions containing APIT purified from crude ink were two absorption maxima at 390 nm and 470 nm, a hallmark of flavoproteins. A flavine nucleoside, particularly FAD is required
20 as a co-factor for the anti-tumor and oxidase activity of APIT as removal of FAD inactivated APIT (example 5).

Analysis of the sequences SEQ ID NO: 2, 4, and 6 revealed that APIT comprises a sequence similar to known dinucleotide binding folds which
25 are characteristic for flavoproteins (Fig. 4c). The GG-motif (consensus sequence RhGGRh_xT/S) is found adjacent to the dinucleotide binding fold.

A further aspect of the present invention is a polypeptide comprising a fragment of the polypeptides of the sequences of SEQ ID NO: 2, 4, or 6
30 which can be used as a lead structure for drug development. APIT can be digested by a protease without loss of activity. Digestion leaves the substrate specificity unaltered. Thus, the fragment exhibiting cytotoxic

activity is an L-lysine and/or L-arginine oxidase. Preferably, proteinase K is used which is a relative unspecific protease resulting in small fragments. Other proteases which can be selected among specific or unspecific proteases known by a person skilled in the art can be used instead of proteinase K. The cytotoxic proteinase resistant domain of APIT is of particular importance for the development of a non-immunogenic, fully active small compound.

Further preferred fragments comprise partial amino acid sequences of APIT which are obtained by peptide mass fingerprinting, ESI/MS, and Edman degradation:

DG(I/V)CRNRRQ (SEQ ID NO: 46),

DSGLDIAVFEYSDR (SEQ ID NO: 47),

VFEYSDR (SEQ ID NO: 48),

LFXYQLPNTPDVNLEI (SEQ ID NO: 49) (X = T in SEQ ID NO: 2, 4 and 6),

VISELGLTPK (SEQ ID NO: 50),

GDVPYDLSPEEK (SEQ ID NO: 39),

VILAXPVYALN (SEQ ID NO: 51) (X = M in SEQ ID NO: 2, 4 and 6),

ATQAYAAVRPIPAK (SEQ ID NO: 37),

VFMTFDQP (SEQ ID NO: 52),

SDALFFQMYD (SEQ ID NO: 53) (FFQ is FSQ in SEQ ID NO: 2, 4 and 6),

SEASGDYILIASYADGLK (SEQ ID NO: 54),

NQGEDIPGSDPQYNQVTEPLK (SEQ ID NO: 55) (PQY is PGY in SEQ ID NO: 2, 4 and 6)

While not wishing to be bound by theory, the FAD group which is tightly bound to the amino acid chain, e.g. by a covalent bond, might cover possible protease cleavage sites. Thus, protease treatment results in a fragment comprising the active centre of the enzyme, including the prosthetic group FAD. This conclusion is confirmed by the finding that native APIT cannot be cleaved by trypsin, but trypsin can digest denaturated APIT.

Thus, an especially preferred fragment of APIT which is an oxidase exhibiting cytotoxic activity is a sequence comprising the dinucleotide binding fold and the GG motif corresponding to amino acid residues No. 39 to 77 in SEQ ID NO: 2. This sequence is identical to the sequence of amino acid residues No. 38 to 76 in SEQ ID NO: 4 and No. 21 to 59 in SEQ ID NO: 6. More preferably, the fragment has an L-lysine and/or an L-arginine oxidase activity.

Further, the fragment can comprise a stretch of additional amino acid residues which may be selected from SEQ ID NO: 2 or 4 from the sequences adjacent to the residues No. 39 to 77 in SEQ ID NO: 2 or No. 38 to 76 in SEQ ID NO: 4. Preferably, 1-20 additional amino acid can be present at the N-terminus and/or the C-terminus. More preferably, 1-10 additional amino acid can be present at the N-terminus and/or the C-terminus. Most preferably, 1-5 additional amino acid can be present.

A further aspect are polypeptides which are homologous to the polypeptides of SEQ ID NO: 2, 4, or 6, or to fragments thereof, which have an identity of at least 70%, preferably at least 80%, more preferably at least 90%, or most preferably at least 95%. SEQ ID NO: 2, 4, or 6 describe natural variations of APIT by replacements of single amino acids not affecting its function. In further 11 clones, four mutations were found within the sequence comprising the dinucleotide binding fold and the GG motif (Pos. 39 to 77 in SEQ ID NO: 2, see example 4). Taking into account that a fragment obtained by proteolytic digestion is still active as a L-lysine and/or L-arginine oxidase, it can be expected that further modifications of the sequence, e.g. by amino acid substitutions, deletions and/or insertions will not substantially affect the function of APIT. A modified sequence exhibits an identity of preferably at least 70%, more preferably at least 80% and most preferably at least 90% to a reference sequence, e.g. SEQ ID NO: 2. Preferably, the sequence of Pos. 39 to 77 in SEQ ID NO: 2 has a higher degree of identity to the reference sequence than the total amino

acid sequence, e.g. preferably at least 33 of 39 amino acid residues (at least about 85%), more preferably 35 of 39 residues (at least about 90%), and most preferably 37 of 39 residues (at least about 95%).

5 A still further aspect is a polypeptide of the present invention as described above which is a recombinant polypeptide. The recombinant polypeptide is characterized as being manufactured in a heterologous, i.e. non-*Aplysia* host cell, e.g. in a bacterial cell such as *E. coli* or *Bacillus*, in a yeast cell such as *saccharomyces cerevisiae*, in an insect cell or in a mammalian cell.

10 The recombinant polypeptide has preferably an oxidase, or, more preferably, an L-lysine and/or an L-arginine oxidase activity. Expression of the polypeptide can be done by standard expression systems known by a person skilled in the art. For proper enzymatic function, the prosthetic group FAD may have to be introduced into the polypeptide.

15 The protein of the invention or a fragment thereof may be in the form of a fusion protein, i.e. fused to heterologous peptide or polypeptide sequences. Preferably fusion proteins are genetic fusions, wherein the nucleic acid sequence encoding a protein or a protein fragment as described above is
20 fused to a nucleic acid sequence encoding a heterologous peptide or polypeptide sequence. The heterologous peptide or polypeptide sequence may be selected from signal sequences, which provide desired processing and/or transport in a host cell. The signal sequence is preferably located at the N- and/or C-terminus of the APIT sequence. Further examples of
25 heterologous sequences are domains which assist expression in host cells and/or purification from cellular extracts or culture media. Still further examples of heterologous sequences are targeting sequences which may direct the APIT polypeptide to a desired target site, e.g. in an organism. Suitable targeting sequences may be e.g. single chain antibodies, which
30 may be directed against tumor specific antigens or proteinaceous ligand sequences, which may be directed against tumor specific receptors.

A further aspect of the present invention is a nucleic acid coding for the polypeptide as described above. The total mRNA of the mantle gland, the nidamental gland, the digestive gland, and the opaline gland can be prepared by standard methods. The mRNA can be reverse transcribed using the tagged oligo dT oligonucleotide (Oligo 1, Fig. 4b). The tag is a

5 random sequence not expected to be present within *Aplysia* mRNA to be reverse transcribed. PCR can be performed using the degenerated primer (Oligo 2) derived from the APIT peptide VFEYSDR and the specific primer (Oligo 3) directed against the tag sequence of the oligo dT primer Oligo 1.
10 The amplified sequence can be cloned into a standard vector and can be sequenced by standard techniques. By this strategy, the 3' terminal sequence of the APIT gene can be obtained. The 5' terminal sequence can be obtained by the RACE strategy. The mRNA from selected tissues (see above) is reverse transcribed using an oligonucleotide derived from the
15 known 3' terminal sequence (e.g. Oligo 4, or Oligo 6) and can be treated with a terminal transferase in the presence of CTP, resulting in a 3'-poly-C-sequence (at the minus strand). PCR can be performed using a tagged primer against the poly-C-sequence (Oligo 5) and a specific primer, e.g. Oligo 4, or Oligo 6. The amplified product can be cloned and
20 sequenced by standard techniques. Finally, for obtaining full-length cDNA clones, specific primers, e.g. Oligo 8 and Oligo 9 can be used. By this strategy, three different clones were obtained and sequenced. The nucleotide sequences are described in SEQ. ID. No.1, 3, and 5 which are identical to 97% (1560 of 1608) of the nucleotides. 42 of 48 mutations
25 are silent mutations which have no effect upon the amino acid sequence.

By this strategy, further clones of APIT can be obtained which might have a differing sequence. Since more than ten sequences of APIT are known, specific or degenerated primers may be selected from these sequences,
30 and new clones can be obtained by a single PCR of reverse transcribed mRNA.

Thus, the nucleic acid encoding a polypeptide as specified above preferably comprises

- (a) a nucleotide sequence as shown in SEQ ID NO: 1, 3, or 5, or at least the polypeptide coding portion thereof, or the complement thereof, or
- (b) a nucleotide sequence corresponding to the sequence of (a) within the scope of degeneracy of the genetic code, or the complement thereof, or
- (c) a nucleotide sequence hybridizing under stringent condition with the sequence of (a) and/or (b), or
- (d) a nucleotide sequence which is homologous to the sequences of (a) and/or (b).

The nucleic acid may be a single stranded or double stranded nucleic acid (DNA or RNA). The nucleic acid is obtainable from natural sources e.g. from *Aplysia* by extraction of RNA, construction of cDNA libraries and screening of the library using degenerated oligonucleotides which were deduced from the peptide sequences described above. The nucleic acid is further obtainable by RT-PCR using RNA extracted from *Aplysia* and oligo-dT-primers or degenerated primers. On the other hand, the nucleic acid is obtainable by chemical synthesis.

Hybridization under stringent conditions preferably means that after washing for 1 h with 1 x SSC and 0.1% SDS at 55°C, preferably at 62°C and more preferably at 68°C, particularly after washing for 1 h with 0.2 x SSC and 0.1% SDS at 55°C, preferably at 62°C and more preferably at 68°C, a hybridization signal is detected.

The degree of identity of the nucleic acid is at least 70%, preferably at least 80%, more preferably at least 90%, and most preferably at least 95% to a reference sequence, e.g. SEQ ID NO: 1, 3 or 5.

Further, the nucleic acid encoding a cytotoxic polypeptide can comprise a partial sequence of the nucleotide sequence as disclosed in SEQ ID NO: 1, 3, or 5. Preferably, the partial sequence is selected from nucleotide No. 115 to 231 in SEQ ID NO: 1, or nucleotide No. 112 to 228 in SEQ ID NO: 3, or nucleic acid residue No. 61 to 177 in SEQ ID NO: 5, or the partial sequence codes for at least one of the eleven fragments of APIT obtained by peptide mass fingerprinting, ESI/MS, and Edman degradation. Further, the partial sequence can comprise a stretch of additional nucleotides selected from the sequences adjacent to the sequence selected from SEQ ID NO: 1, 3, or 5. Preferably, 1-60 additional nucleotides can be present at the 5' and/or the 3'-terminus. More preferably, 1-30 additional nucleotides can be present at the 5' and/or the 3'-terminus. Most preferably, 1-10 additional nucleotides can be present at the 5' and/or the 3'-terminus.

Furthermore, the nucleic acid may encode a fusion polypeptide as described above.

In a preferred embodiment of the invention the nucleic acid is operatively linked to an expression control sequence, e.g. a sequence which is capable of directing expression in a suitable host cell, e.g. a prokaryotic or eukaryotic host cell. The expression control sequence usually comprises a promoter and optionally operator or enhancer sequences which enable a transcription of the nucleic acid operatively linked thereto. Furthermore, the expression control sequence may contain a translation signal, e.g. a ribosome binding sequence.

The nucleic acid of the present invention may be a recombinant vector which contains in addition usual vector sequences such as an origin of replication, a selection marker gene and/or a cloning site. Examples of suitable vectors such as plasmids, phages or viral vectors are known to the skilled person and are described e.g. in Sambrook et al., Molecular Cloning,

A Laboratory Manual (2nd ed. 1998), Cold Spring Harbor, Laboratory Press.

5 A further aspect of the present invention is a recombinant cell transformed or transfected with a nucleic acid as described above. The recombinant cell may be a prokaryotic cell, e.g. a gram-negative prokaryotic cell such as *E. coli* or an eukaryotic cell, e.g. an insect cell or a vertebrate cell such as a mammalian cell. Techniques for transforming or transfecting host cells with nucleic acids are known to the skilled person and e.g. described in
10 Sambrook et al., supra.

Still a further subject matter of the present invention is an antibody directed against the polypeptide as described above. The antibody may inhibit the cytotoxic activity of the polypeptide. The antibody may be a
15 polyclonal or monoclonal antibody or a recombinant antibody, e.g. a chimeric antibody, a humanized antibody or a single chain antibody. Furthermore, the antibody may be an antibody fragment containing the antigen-binding site of the antibody, e.g. a Fab fragment. The antibody may be obtained by immunizing suitable experimental animals with an
20 *Aplysia* polypeptide as described above or a partial fragment thereof or a peptide antigen optionally coupled to a suitable macromolecular carrier according to known protocols, e.g. by techniques which are described in Borrebaeck, Carl A.K. (Ed.), Antibody engineering (1992), or Clark, M. (Ed.), Protein engineering of antibody molecules for prophylactic and
25 therapeutic applications in man (1993). By techniques for producing hybridoma cell lines according to Köhler and Milstein monoclonal antibodies may be obtained.

30 Methods for introducing a prosthetic group into a polypeptide are known in the art. Preferably, the FAD is introduced by a method comprising surface display of the polypeptide on a prokaryotic host, comprising the steps:

- (a) providing a prokaryotic host cell transformed with a nucleic acid fusion operatively linked with an expression control sequence, said nucleic acid fusion comprising sequences necessary for displaying the protein on the outer membrane, and
- 5 (b) culturing the host cell under condition wherein the nucleic acid fusion is expressed and the expression product comprising the recombinant polypeptide is displayed on the surface of the host cell, and
- 10 (c) contacting the recombinant polypeptide with FAD under conditions wherein FAD combines with the recombinant polypeptide and a functional recombinant polypeptide containing the prosthetic group is formed.

15 The nucleic acid fusion may be formed using a nucleic acid sequence as described above and further sequences necessary for surface display. Details describing the prokaryotic host cells, the sequences necessary for surface display of the polypeptide, culture conditions, and the conditions under which the recombinant polypeptide is contacted with FAD are described in WO 02/070645, which is included by reference herein.

20

A further aspect of the present invention relates to diagnostic or therapeutic applications in humans or animals. The polypeptide, and/or a nucleic acid, and/or a recombinant cell, and/or an effector, e.g. an inhibitor or activator of the polypeptide as described above can be used in such applications. The polypeptide as described above is able to selectively kill tumor cells. For example, T and B leukemia cell lines, a chronic myeloid leukemia cell line (K562), cells from an orphan and aggressive osteosarcoma (Ewings tumor: RDES, A673), a small cell lung cancer cell line (GLC4, GLC4/ADR), cervix cancer (Chang), and acute monocytic leukemia (THP-1) show an $IC_{50} \leq 10$ ng/ml APIT.

25

30

Healthy human cells are resistant against APIT-induced cell death. At a concentration of 40ng/ml, APIT induces a cell death below 10% in normal HUVEC cells (Example 13). This indicates that the APIT IC_{50} values of healthy cells are at least one order of magnitude higher than the IC_{50} of tumor cells.

Resistance to apoptosis as well as multi drug resistance (MDR) represent severe problems in cancer therapy. It is therefore of particular interest that the polypeptide of the present invention kills apoptosis resistant cell lines as well as MDR cancer cell lines to the same extent as their non resistant counter parts. Over-expression of apoptosis inhibitors of the Bcl-2 family in cancer cell lines does not protect from APIT mediated cell death, confirming that APIT induces cell death in an apoptosis independent way. The MDR cell line GLC4/ADR possess almost the same sensitivity to APIT (IC_{50} 10 ng/ml) as the parental cancer line GLC4 does (IC_{50} 9 ng/ml).

Thus, the diagnostic or therapeutic application preferably relates to a method for diagnosis or treatment of hyperproliferative diseases, e.g. cancer. More preferably, the method is a method for diagnosis or treatment of lung cancer, breast cancer, prostate cancer, colon cancer, cervix cancer, uterus cancer, larynx cancer, stomach cancer, liver cancer, Ewings sarkoma, acute lymphoid leukemia, acute and chronic myeloid leukemia, apoptosis resistant leukemia, and/or MDR lung cancer. Moreover other tumor types can also be treated with the polypeptide, like pancreas cancer, gastric cancer, kidney cancer, gliomas, melanomas, chronic lymphoid leukemia, and/or lymphoma. Since all cancer cell lines tested (in total 24) were effectively killed by APIT, the polypeptide can be used for the treatment of solid tumors and leukemias in general including apoptosis resistant and multi drug resistant cancer forms.

A further aspect of the present invention is a pharmaceutical composition comprising the polypeptide of the present invention as described above, in

a pharmaceutically effective amount and optionally together with suitable diluents and carriers or kit containing the composition together with other active ingredients, e.g. modulators of the polypeptide or other cytostatic or cytotoxic agents. The composition can be administered locally or
5 systemically by any suitable means, e.g. orally, nasally or by injection (i.v., i.p., s.c., or i.m.) to a subject in need thereof. The components of a kit, which consists of at least two different compositions may be administered together or separately, e.g. at different times and/or by different routes.

10 In another embodiment, the pharmaceutical composition or the kit comprises a nucleic acid encoding for the polypeptide of the present invention as described above. Further, the pharmaceutical composition or kit may comprise both the polypeptide and the nucleic acid of the present invention.

15 From many studies it is known that tumor cells have an increased rate of metabolism compared to normal cells. A result of this high metabolic rate is a high concentration of reactive oxygen species (ROS, comprising H_2O_2) which originate from oxidative phosphorylation reactions by the electron
20 transport chain of the mitochondria. As a consequence ROS detoxification reactions are increased in tumor cells, and interference with detoxification has a selective toxic effect on the tumor cells but not on normal cells. Likewise, increasing the concentration of H_2O_2 by administering the polypeptide of the invention in a predetermined amount may overcome the
25 detoxification reactions and kill the tumor cells. The level of extra H_2O_2 produced by exogenous APIT does not affect normal cells because of their higher tolerance for additional H_2O_2 . An administration of the polypeptide in a varying amount, e.g. a gradually changing, e.g. increasing amount leads to the production of a defined amount of H_2O_2 could thus be used for
30 a selective killing of cancer cells.

The pharmaceutical composition or kit as described above can comprise a further component which is a substance capable of modulating the cytotoxic activity of the polypeptide, in a pharmaceutically effective amount and optionally together with suitable diluents, and carriers. In FCS (100%) at 37°C and 5% CO₂ which reflect *in vivo* conditions, or in a medium containing 10% FCS (typical *in vitro* conditions) devoid of L-lysine and L-arginine, the activity of APIT (20 ng/ml) can be dose-dependently increased by the addition of L-lysine in a final concentration of 2 – 50 µg/ml. Thus, the high specificity of APIT for L-lysine (and L-arginine) allows for modulating the enzymatic activity of APIT and thus its cytotoxic activity by providing an additional substrate *in vivo* or *in vitro*. The substance capable of modulating the cytotoxic activity of the polypeptide can be L-lysine, L-arginine, a derivative or metabolic precursor of L-lysine, or L-arginine, or a mixture thereof. A derivative is a compound which is an APIT substrate. A metabolic precursor is a compound, which can be metabolized to a compound, which is an APIT substrate. Further, the modulator may be selected from flavine nucleosides, particularly FAD, since the presence of a flavine nucleoside prosthetic group leads to a great increase in APIT activity.

The pharmaceutical composition may comprise the polypeptide and at least one modulating substance as a mixture. Preferably, the modulating substances are provided in a kit consisting of separate preparations. More preferable, the polypeptide is provided for administration before the modulating substances.

During the passage through body fluids before reaching the tumor tissue, the cytotoxic activity of the polypeptide would be undesired, due to the toxic properties of H₂O₂. Thus, the composition may further comprise an inhibitor of the polypeptide. The inhibitor could have a short half-life time in the body fluid. A preferred inhibitor of the polypeptide is an antibody against the polypeptide (see above).

Further the polypeptide can be coupled with a substance and/or a particle which targets the polypeptide to the tumor tissue.

Further components of the pharmaceutical composition can be a nucleic acid coding for the polypeptide as described above, and/or a recombinant vector or cell containing the nucleic acid.

A further aspect of the present invention is a substance modified by interaction with APIT (termed target substance of APIT). A direct interaction is a contact of APIT with this substance. In an indirect interaction, the effect upon the substance includes at least one mediator substance, e.g a substance formed by APIT, or a receptor interacting with APIT and the components of the related transduction cascade.

As described above, a mediator of APIT acting on cellular polypeptides is H_2O_2 . Thus, preferred target substances of APIT comprise cellular polypeptides, which can be modified by H_2O_2 . A major modification identified in 2-DE SDS gel patterns of cells treated with APIT was a shift of peroxiredoxin I (Prx I, Swiss-Prot No. Q06830, Genbank identifier No. 548453, SEQ ID NO: 8), which was also detected in cells treated with H_2O_2 . Prx I belongs to a class of peroxidases which are involved in the detoxification of ROS. Although the nature of the modification of Prx is not known, Prx I can be used as a marker for APIT anti-tumor activity.

Thus, particularly preferred substances which can be used as target substances of the polypeptide as described above are peroxidases, especially preferably peroxiredoxin I or a polypeptide having substantially the same biological activity as peroxiredoxin I. Peroxiredoxin I may comprise

(a) the amino acid sequence shown in SEQ ID NO: 8, or/and

- (b) an amino acid sequence which is homologous to the sequence of (a) with at least 70%, preferably 80%, particularly preferably 90%, especially preferably 95%, or/and
- (c) a fragment of the amino acid sequence of (a) or (b).

5

Further, peroxiredoxin I may comprise an amino acid sequence or a fragment thereof as disclosed in at least one of the Genbank entries selected from gi:4505591 (NP_002565.1), gi:13626803 (XP_001393.2), gi:32455264 (NP_859047.1), gi: 32455266 (NP_859048.1), gi: 423025 (A46711), gi: 287641 (CAA48137.1), gi: 13937907 (AAH07063.1), gi: 18204954 (AHH21683.1) or gi:440306 (AAA50464.1).

10

WO 02/31144 discloses proteins modified by H_2O_2 which are targets of APIT: thioredoxin peroxidase 2 (Swiss Prot No. Q06830, Genbank identifier 548453), 60S ribosomal protein P0 (12654583), Hsp-60 (N-term) (14603309), stathmin (5031851), Rho GDI 2 (P52566, 1707893), 60S ribosomal protein P0(4506667), RNA binding regulatory subunit (O14805,12720028), hnRNP C1/C2 (4758544), hnRNP C1/C2 (4758544), proteasome subunit beta type 1 (P20618, 130853), pre-mRNA cleavage factor Im (5901926), proteasome subunit alpha type 7 (O14818, 12643540), U2 small nuclear ribonucleo-protein A' (P09661, 134094), GAP SH3 binding protein (5031703), DNA replication licensing factor MCM4 (P33991, 1705520), thioredoxin peroxidase 1 (P32119, 2507169), 40S ribosomal protein S21 (P35265, 464710), 40S ribosomal protein S12 (P25398, 133742), phosphoglycerate mutase 1 (P18669, 130348), HCC-1 protein (13940310), HnRNP A2/B1 (4504447/14043072), IMP dehydrogenase 2 (P12268, 124419), hnRNP A/B (14724990).

20

25

30

Further targets of APIT identified by 2 DE gel electrophoresis, in-gel tryptic digestion, peptide mass fingerprinting by MALDI-MS, and identification of the proteins are summarized in Table 3.

Still a further target of APIT is a nucleic acid. The target nucleic acid can be a DNA or an RNA, which is a mRNA. The transcription of the mRNA is up- or downregulated in the presence of APIT and/or H₂O₂. Preferably, the transcription is changed by a factor of at least 2, and more preferably, by a factor of at least 4.

By a microarray of specific 60mer oligonucleotides representing about 8500 human genes, about 70 mRNAs were identified which are targets of APIT. The information about the mRNAs are summarized in Table 4. Each mRNA is referenced by a "unigene cluster" which represents a number of nucleotide sequences belonging to the same gene or to closely related genes. Details of the nomenclature and the nucleotide sequences of the unigene clusters are public available under <http://www.ncbi.nlm.nih.gov/> (Homepage of the National Center for Biotechnology Information).

For most of the unigene clusters of Table 4, the gene and/or the protein is known. It is a general principle that modulation of the transcription of a messenger RNA influences the amount of protein expressed. Thus, the proteins coded by the sequences of the unigene clusters of Table 4 are also targets of APIT, because APIT may influence their expression. The sequences of the proteins and of the nucleic acids coding for these proteins are referenced by the genbank identifier, accession number and/or version number (see Table 4). The sequences are public available under <http://www.ncbi.nlm.nih.gov/>.

Additional targets of APIT (nucleic acids, proteins) obtained by microarray analysis as described above are summarized in Table 5.

A preferred substance which can be used as a target substance for the polypeptide as described above is a nucleic acid coding for a peroxidase, particularly preferably peroxiredoxin I or a polypeptide having substantially

the same biological activity as peroxiredoxin I. The nucleic acid coding for peroxiredoxin I may comprise

- (a) the nucleotide sequence shown in SEQ ID NO: 7, or/and
- (b) a nucleotide sequence which corresponds to the sequence of
5 (a) within the scope of the degeneracy of the genetic code,
or/and
- (c) a nucleotide sequence hybridizing to the sequence of (a)
or/and (b) under stringent conditions, or/and
- (d) a fragment of the nucleotide sequence of (a), (b) or (c).

10 SEQ ID NO: 7 is disclosed in Genbank entry gi:14721336 (XM001393).

Preferably, the nucleic acid encoding peroxiredoxin I may comprise a
nucleotide sequence which is homologous to SEQ ID NO: 7 with at least
15 70%, particularly preferably at least 80%, especially preferably at least
90%.

In further preferred embodiments, the nucleic acid encoding peroxiredoxin
I may comprise a nucleotide sequence or a fragment thereof as disclosed
20 in at least one of the Genbank entries selected from gi: 13937906
(BC007063.1, PRDX1 transcript 3), gi: 18204953 (BC021683.1, PRDX1
transcript variant 3), gi: 32455265 (NM_181697.1, PRDX1 transcript
variant 3), gi: 34528302 (AK131049.1, clone highly similar to PRDX1), gi:
287640 (X679851.1, PAG), gi: 32455263 (NM_181696.1, PRDX1
25 transcript variant 2), gi: 32455267 (NM_002574.2, PRDX1 transcript
variant 2) or gi:440305, (L19184, NKEF A).

The target substance of the present invention (see Table 3, 4 and 5),
which is identified by one of the methods as described above, may be used
30 for the development of new pharmaceutical agents, e.g. by known
high-throughput screening procedures which may be cellular screening
procedures or molecular based screening procedures. These pharmaceutical

agents may act upon cellular receptors and/or components of the signal transduction pathways activated or inhibited by APIT.

Degenerative diseases like Alzheimer's and Parkinson's disease are characterised by excessive ROS production of the affected tissue. Drugs which either activate H_2O_2 detoxification or inhibit H_2O_2 production may be used for therapy of degenerative diseases like Alzheimer's or Parkinson's disease. Fast growing tumor cells produce more ROS and thus require an efficient H_2O_2 detoxification system. Drugs which either activate H_2O_2 production or which interfere with H_2O_2 detoxification may be used for therapy of proliferative diseases like tumors. Since e.g. thioredoxin peroxidases 1 and 2 have been shown to be overexpressed in cells at risk for diseases related to ROS toxicity including degenerative diseases like Alzheimer's and Parkinson's disease, and have been shown to be overexpressed in tumor cells (Butterfield et al., 1999, *Antioxidants & Redox Signalling*, 1, 385-402), the targets of Table 3 and 4 might be important targets for the development of drugs for treatment of degenerative diseases like Alzheimer's and Parkinson's disease and of proliferative diseases like tumors.

NK-cells have been shown to protect against malignant cells in chronic myelogenous leukemia (CML), but their number and inducibility is reduced during the progression of the disease. This reduction and dysfunction is due to the production of H_2O_2 by CML-cells (Mellqvist, Blood 2000, 96, 1961-1968). NK-cells encountering H_2O_2 are inhibited in their lytic activity, are made resistant to IL-2 activation and undergo apoptosis/necrosis. Any therapy providing CML-patients with ROS-hyposensitive NK-cells therefore would be of great benefit. The targets described above could be used to modulate the H_2O_2 sensitivity of NK-cells or to inhibit the H_2O_2 production of malignant cells, e.g. CML-cells.

Arteriosclerosis with its progression to heart disease, stroke and peripheral vascular disease continues to be the leading cause of death in all western civilisations. Enhanced ROS-production (via endothelial NADPH-oxidase) is required and sufficient to generate the pathologic phenotype (Meyer, FEBS Letters 2000, 472, 1-4). Therefore, targets mediating the effect of H_2O_2 are useful to develop new drugs for treatment of arteriosclerosis and the associated diseases like heart disease, stroke and other vascular diseases. These targets are suitable to detoxify H_2O_2 and/or to block the H_2O_2 induced signalling pathways.

Target compounds, e.g. peptides, polypeptides or low-molecular weight organic compounds, which are capable of modulating the effect of H_2O_2 may be identified in a screening system comprising the use of the APIT polypeptide as described above. Particularly, a modulation of the APIT activity, i.e. L-amino oxidase activity, may be determined.

Thus the present invention further relates to a pharmaceutical composition comprising as an active agent at least one of the target substances as described above.

Still a further aspect of the present invention is an inhibitor of a target as described above, in particular an inhibitor of the detoxification system of the cell which eliminates reactive oxygen species, e.g. H_2O_2 . Surprisingly, it was found that the inhibition of detoxifying enzymes sensitized tumor cells to the cytotoxic activity of the polypeptide of the present invention as described above. Example 11 demonstrates that knock-down of peroxiredoxin I sensitized tumor cells for APIT-induced cell death.

Preferably, the inhibitor is an inhibitor of peroxidase, particularly of peroxiredoxin I. The inhibitor may be an antibody or a nucleic acid molecule, i.e. useful for antisense inhibition or as an siRNA molecule. It is particularly preferred that the inhibitor is an inhibitor of peroxiredoxin I

activity which is an RNA molecule, particularly a double-stranded RNA molecule comprising a nucleic acid sequence of at least 15 nucleotides complementary to a peroxiredoxin I transcript. It is especially preferred that the peroxiredoxin I transcript is derived from SEQ ID NO:7.

5

The one or two strands of the RNA molecule as described above may, independently, have a length of 19 to 25 nucleotides, preferably 19 to 23 nucleotides. Especially preferred is a length of the one or two strands of 19, 20, 21, 22 or 23 nucleotides. The RNA molecule as described above
10 may comprise at least one modified nucleotide. Preferably, modified nucleotides are selected from the group consisting of oxetane[1-(1',3'-O-anhydro- β -D-psicofuranosyl)-nucleotides, locked nucleic acid (LNA) nucleotides, hexitol nucleotides, altritol nucleotides, cyclohexane nucleotides; neutral phosphate analogs.

15

The double-stranded RNA molecule as described above may have one or two 3' overhangs with, independently, a length of 1 to 5 nucleotides, preferably 1 to 3 nucleotides, particularly preferably 2 nucleotides. The one or two overhangs may consist of ribonucleotides, deoxyribonucleotides,
20 modified nucleotides as described above or combinations thereof.

The double-stranded RNA molecule as described above may comprise a sequence selected from the group of sequences consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11; SEQ ID NO: 12; SEQ ID NO: 13; SEQ
25 ID NO: 14; SEQ ID NO: 15; SEQ ID NO: 16; SEQ ID NO: 17; SEQ ID NO: 18; SEQ ID NO: 19; SEQ ID NO: 20; SEQ ID NO: 21; SEQ ID NO: 22; SEQ ID NO: 23; SEQ ID NO: 24; SEQ ID NO: 25; SEQ ID NO: 26; SEQ ID NO: 27; SEQ ID NO: 28; SEQ ID NO: 29.

30 Yet another aspect of the present invention is a pharmaceutical composition or kit comprising an inhibitor as described above, preferably an RNA molecule, particularly preferred a double-stranded RNA molecule, or a

nucleic acid encoding such an RNA molecule. The pharmaceutical composition or kit may comprise the inhibitor as sole active agent in order to increase the amount of reactive oxygen species present in the cell due to endogenous production. More importantly, the pharmaceutical composition or kit may comprise the inhibitor and a substance capable of producing reactive oxygen species. In a preferred embodiment, the pharmaceutical composition or kit comprises as an active agent a combination of APIT and at least one inhibitor of a target substance as described in Table 3 or/and Table 4 or/and Table 5, more preferably at least one inhibitor of peroxiredoxin I. In another preferred embodiment, the pharmaceutical composition or kit comprises at least one inhibitor of a target substance as described in Table 3 or/and Table 4 or/and Table 5, more preferably at least one inhibitor of peroxiredoxin I, and the polypeptide of the present invention having cytotoxic activity as described above. In yet another preferred embodiment, the pharmaceutical composition or kit comprises at least one inhibitor of a target substance as described in Table 3 or/and Table 4 or/and Table 5, more preferably at least one inhibitor of peroxiredoxin I, and a cytotoxic polypeptide producing reactive oxygen species or/and a nucleic acid encoding such a cytotoxic polypeptide, wherein the cytotoxic polypeptide is selected from cytotoxic polypeptides obtainable from sea hares, e.g. Cyplasin C, Cyplasin L, Aplysianin A, Aplysianin P, Aplysianin E, Dolabellin A, Dolabellin C, Dolabellin P, Julianin G, Julianin S, or is selected from L-Lysine oxidases like EC 1.4.3.14 from Trichoderma, AIP from Chub mackerel (AJ400871), Apoxin from Crotalus (AAD45200.1), or from other L-amino acid oxidases like EC 1.4.3.2 or from other enzymes which produce H_2O_2 . More preferably, the pharmaceutical composition or kit comprises

(I) a polypeptide obtainable from *Aplysia* comprising an amino acid sequence selected from:

- (a) D-G-E-D-A-A-V (SEQ ID NO:32) and/or
- (b) (D/Q)-G-(I/V)-C-R-N-(Q/R)-R-(Q/P) (SEQ ID NO:33),
- (c) F-A-D-S (SEQ ID NO:34),

- (d) G-P-D-G-(I/L)-V-A-D (SEQ ID NO:35),
- (e) P-G-E-V-S-(K/Q)-(I/L) (SEQ ID NO: 36),
- (f) A-T-Q-A-Y-A-A-V-R-P-I-P-A-S-K (SEQ ID NO:37),
- (g) D-S-G-L-D-I-A-V-E-Y-S-D-R (SEQ ID NO:38),
- (h) G-D-V-P-Y-D-L-S-P-E-E-K (SEQ ID NO: 39) or/and

(i) SEQ ID NO: 41, 43, 44, 45.

or a fragment thereof wherein the polypeptide or the fragment has cytotoxic activity, or/and a nucleic acid encoding the cytotoxic polypeptide obtainable from *Aplysia* comprising

- (i) a nucleotide sequence as shown in SEQ ID NO:40 or 42 or at least the polypeptide coding portion thereof or the complement thereof,
- (ii) a nucleotide sequence corresponding to the sequence of (a) within the scope of degeneracy of the genetic code, or the complement thereof, or/and
- (iii) a nucleotide sequence hybridizing under stringent conditions with the sequence of (a) or/and (b), and

(II) an inhibitor of a target substance as described in Table 3 or/and Table 4 or/and Table 5.

The inhibitor of the present invention may be coupled to carriers, (e.g. lipids, peptides, biodegradable polymers, dendrimers, vitamins, carbohydrate receptors) for *in vivo* targeting to predetermined tissues or/and cell types.

Delivery of the inhibitors of the present invention may be improved by linking the inhibitors with lipids, liposomes, PEG, nanoparticles or/and polymers, for example.

Yet another aspect of the present invention is a gene therapy delivery system suitable for delivery of a nucleic acid encoding an inhibitor which is an RNA molecule, preferably a double-stranded RNA molecule as described above, capable of inhibiting peroxidase, particularly peroxiredoxin I activity.

5 Suitable delivery systems for gene therapy are commonly known in the art, for instance a recombinant adenoviral delivery system, a recombinant adenoviral-derived system or a recombinant lentiviral system. Further, the nucleic acid may be delivered by virus-like particles from *Papillomaviridae* and *Polyomaviridae*. Further, bacteria may be used as a delivery system,
10 e.g. attenuated gram negative bacteria, particularly attenuated salmonella strains. The nucleic acid encoding the inhibitor is operatively linked with expression control sequences which are adapted to the host and to the delivery system. Such expression control sequences are known to a person skilled in the art. Expression of the two strands of the RNA molecule may
15 be performed together in a self-complementary configuration which allows formation of a small hairpin RNA (shRNA) in which the two strands of the double-stranded molecule are interconnected by an additional loop, or may be performed as two separate strands which hybridize later on in the host.

20 Yet another aspect is a pharmaceutical composition or kit comprising a delivery system suitable for delivery of a nucleic acid encoding an inhibitor which is an RNA molecule, particularly a double-stranded RNA molecule preferably comprising a nucleic acid of at least 15 nucleotides complementary to a peroxiredoxin I transcript as described above, to
25 predetermined tissues or/and cell types.

In yet another embodiment, the invention concerns a method for diagnosis or treatment of cancer, wherein a pharmaceutical composition as described above is administered to a subject in need thereof.

30 SEQ ID NO: 1, 3 and 5 show the APIT nucleotide sequences as shown in Fig. 4c. SEQ ID NO: 2, 4 and 6 show the amino acid sequences derived

from SEQ ID NO: 1, 3 and 5, respectively. SEQ ID NO: 7 and 8 show the nucleotide sequence and the amino acid sequence of Prx I. SEQ ID NOs: 9 to 29 show the nucleotide sequences of double-stranded siRNA molecules capable of inhibiting Prx I activity. SEQ ID NOs: 30 and 31 show sequences of double stranded siRNA molecules obtained from the Lamin

AC and the luciferase sequence, respectively. SEQ ID NOs: 32 to 39 show the amino acid sequences of fragments of cytotoxic *Aplysia* polypeptides. SEQ ID NO: 40 and 42 show partial sequences of nucleic acids encoding cytotoxic polypeptides of *Aplysia punctata*. SEQ ID NOs: 41, 43, 44 and 45 show the derived amino acid sequences of SEQ ID NOs: 40 and 42. SEQ ID NOs: 46 to 55 show the amino acid sequences of fragments of cytotoxic *Aplysia* polypeptides.

The invention is explained in more detail by the following figures, tables and examples.

Figure 1

A, Anion exchange chromatography. Filtrated and concentrated ink was loaded onto a Source Q15 column. Proteins were eluted by a linear gradient from 0 to 800 mM NaCl, fractions were collected every minute (2 ml/min). Absorption was measured at 280 nm. Horizontal bar indicates active fractions.

B, Gelfiltration. Active fractions from the Source Q15 were pooled and concentrated and applied to a Superose 12 HR 10/30 column. Proteins were eluted with 100 mM potassium phosphate buffer (pH 7.2). Fractions were collected every minute (0.5 ml/min). Horizontal bar indicates active fractions.

Figure 2

A, Phenotype of APIT-induced cell death. Jurkat cells were cultured for 7 hours in the presence (APIT) or absence (medium) of APIT (30 ng/ml) and phase contrast images were recorded.

B, Lack of apoptotic DNA fragmentation in ink-treated cells. Jurkat cells were incubated in medium (control) or treated with cycloheximide (chx; 10 μ g/ml) or ink (ink, 1/500 diluted) for 2, 4 and 6 h. Isolated DNA was visualized on a 1,6% agarose gel by ethidium bromide staining.

C, APIT mediated loss of metabolic activity. APIT (10 ng/ml) and the tetrazolium-salt WST-1 were added simultaneously to Jurkat cells and turnover of WST-1 was measured photometrically. White circles: medium control; black circles: APIT-treated samples; mean absorbance of 8 replicates \pm SD.

D, Cell death induced by ink. Jurkat cells were treated with ink (1/500 diluted) and propidium iodide (PI) uptake was measured as indicator for dead cells.

Figure 3

A, Heat sensitivity of ink. Dialysed ink was incubated for 10 min at the indicated temperatures and enzymatic activity was measured as H_2O_2 -production (mean of triplicates \pm SD). Blank: medium control.

B, pH-sensitivity of APIT. APIT (60 ng) was incubated for 10 min at 25 °C in 0,1 M potassium phosphate at indicated pH values. Enzymatic activity was measured as H_2O_2 -production (mean of triplicates \pm SD).

C, Sensitivity to increasing amounts of urea: Dialyzed ink (black bars, 1/500 diluted) and as positive control 0,625 mM α -keto isocaproic acid (open bars) were treated with indicated concentrations of urea for 30 min at 25°C. Enzymatic activity (15 min, 25°C) was measured as α -keto acid formation via MBTH.

Figure 4

A, N-terminal and internal peptide sequences of the APIT protein.

B, List of oligonucleotides used for cloning of the APIT gene.

C, Nucleotide sequence of the APIT cDNA and the derived amino acid sequence. The dinucleotide binding fold (VAVVGAGPGGANSAYMLRDSGLDIAVFE) and the GG-motif (RVGGRLFT) are indicated by boxes. Consensus amino acid residues are indicated by bold letters. The N-terminal sequence of mature APIT (dashed line) and of internal peptides (solid line) derived by Edman degradation and mass finger prints are indicated. Sequence variations of the three clones are indicated by small boxes.

D, Variation of the N-terminus of APIT in 11 further clones.

Figure 5

A, Anion exchange chromatography of purified APIT. Proteins were eluted by a linear gradient from 0 to 800 mM NaCl and fractions were collected every minute. Absorption was measured at 280 nm (AU: Absorption unit).

B, Fractions 24, 27 and 29 were separated by SDS-PAGE and tested for metabolic activity by WST-1 assay. High activity (+; + +) correlated with the presence of a prominent 60 kDa band (fractions 24 and 29). Activity is

given as the dilution leading to > 85% reduction of the metabolic activity of Jurkat cells (+/- = 1:900; + = 1:2700; ++ = 1:8100).

5 C, Absorption spectra of fractions 24 (black line), 27 (dashed line) and 29 (dotted line).

Figure 6

10 A, APIT induced H_2O_2 production in medium in the absence of cells. APIT (260 ng/ml) was incubated in medium in the presence (open bar) or absence (black bar) of Jurkat cells (5×10^5 /ml). After 1 h of incubation at 37°C supernatants were alkylated with N-ethylmaleimide and H_2O_2 was measured (mean values of 3 independent experiments +/- SD).

15 B, Catalase inhibits ink induced cell death. Jurkat T-cells were incubated for 8 h with ink in the presence (black bars) or absence (white bars) of catalase. Cytotoxicity was measured as PI uptake (mean of triplicates \pm SD).

20 C, Catalase protects from APIT induced loss of metabolic activity. Metabolic activity of Jurkat cells was measured after incubation with APIT (20 ng/ml) or anti-CD95 for 3h in the presence (black bars) or absence (white bars) of catalase. (mean of 5 replicates \pm SD).

25 D, Phenotype of APIT induced cell death is mediated by hydrogen peroxide. Jurkat cells were cultured for 7 hours in the presence (APIT) or absence (medium) of APIT (60 ng/ml) or H_2O_2 (500 μ M) and were analyzed by phase contrast microscopy. Catalase was added in combination with APIT to neutralize H_2O_2 (APIT + CAT).

Figure 7

A, Enzymatic activity of APIT in the presence of different medium supplements. APIT (200 ng/ml) was incubated for 60 min at RT with RPMI + /- 10% FCS or KRG supplemented with different medium ingredients and H_2O_2 production was measured. (EAA = essential amino acids, NEAA = non essential amino acids, concentrations see Table 1).

B, Substrate specificity of APIT and ink. The enzymatic reaction of dialysed ink (open bars) with different L-amino acids in potassium phosphate buffer was measured as H_2O_2 -production. 50 μM H_2O_2 and amino acid free medium (control) were used as control. Aliquots of dialyzed ink were digested with trypsin (hatched bars) or proteinase K (black bars) at 37 °C for 2h prior to testing the substrate specificity. Arg = L-arginine, 1mM; Lys = L-lysine, 1mM; EAA = essential amino acids, 1mM; NEAA = non essential amino acids, 1mM.

C, APIT induced cell death depends on the presence of L-lysine or L-arginine. Jurkat cells were incubated with APIT (20 ng/ml) for 6 h in the presence (white bars) or absence of L-lysine and L-arginine (black bars). Cytotoxicity was measured as PI uptake (mean of triplicates \pm SD).

D, APIT induced loss of metabolic activity depends on the presence of L-lysine or L-arginine. Jurkat cells were incubated with APIT (20 ng/ml) or anti-CD95 (150 ng/ml) in the presence (open bars) or absence (black bars) of L-lysine or L-arginine and metabolic activity was measured (mean of 5 replicates \pm SD).

E, APIT transforms L-lysine into an α -keto acid. APIT was incubated with L-lysine and the formation of α -keto acid was measured photometrically by its reaction with MBTH.

F, Michaelis-Menten kinetic of APIT activity with L-lysine. K_m value for L-lysine was determined as H_2O_2 production.

G, Proposed reaction mechanism of L-amino acid oxidases according to Macheroux et al. (2001 Eur. J. Biochem. 268:1679-1686). Encircled are compounds which we demonstrated to participate in the reaction catalyzed by APIT.

Figure 8

A, Quantification of the mRNAs of Lamin A/C and Prx I after transfection of specific siRNA (open bars) and control Luciferase siRNA (black bars) with quantitative realtime PCR. Shown are the relative mRNAs levels compared to the mRNA of GAPDH measured in the same RNA preparation.

B, Sensitization of HeLa cells by knock down of Prx I. Specific siRNAs directed against the mRNA of Luciferase (Luc, transfection control), Lamin A/C (control knock down) and Prx I were transfected in HeLa cells and the metabolic activity of transfectants treated in the presence (black bars) or absence of APIT (open bars) was measured. Note that the knock down of Prx I but not of the other genes sensitized cells for the cytotoxic activity of APIT.

Figure 9

APIT did not induce actin depolymerisation in HeLa cells. Untreated HeLa cells (A) and HeLa cells treated with Cytochalasin (B) or APIT (C) were stained with Phalloidin-TRITC for actin and Hoechst 33258 for nuclei staining. Subsequently, fluorescence microscopy was performed. Actin staining is shown in bright white, nuclei are displayed in transient grey.

Figure 10

HUVEC cells are resistant to the APIT induced cells death. HUVEC and Jurkat cells were incubated with APIT over night and subsequently LDH release in the culture supernatant was measure photometrically. Shown are the results of two independent experiments +/- standard deviation.

Table 1

Composition and concentrations of mixtures of essential and non-essential amino acids as well as single amino acids used in Fig. 7A.

Table 2

APIT kills different kinds of tumor cells. Different tumor cell lines (50,000 cells in 100 μ l) were incubated for 14 h in the presence of increasing amounts of APIT. Metabolic activity of the cells was measured via turnover of WST. The IC₅₀ values reflect the APIT concentration at which the metabolic activity is decreased to 50%. (* stands for IC₅₀ \geq 20 ng/ml at the given cell concentration of 50,000/100 μ l.)

Table 3

List of proteins which were changed in their expression or modified after treatment with APIT (upregulation (+), downregulation (-), or modification (m) in column "effect"). The proteins are referenced by the genbank identifier and/or accession number and/or version number.

Table 4 and Table 5

List of genes (referenced by unigene cluster number) and gene products (proteins) which were modulated in their expression more than 2 fold after incubation with APIT for two hours. The proteins are referenced by the genbank identifier and/or accession number. Transcription rates are indicated as increase (+, 2 to \leq 4 times; ++, 4 to 6 times in Table 4 or

4 to 25 times in Table 5) or decrease (-, 2 to \leq 4 times; --, 4 to 6 times).

Example 1: Purification of APIT

5 *Aplysia punctata* were gained from the Station Biologique Roscoff, Bretagne, France. Crude ink was prepared by gentle squeezing the sea hares in sterile seawater. Insoluble particles were removed by ultracentrifugation (82,000g, 30 min, 4°C) and supernatants were stored at -70°C.

10 APIT was purified from crude ink via anion exchange chromatography and gelfiltration. The thawed ink was filtered through Whatman filter No. 4 under slight vacuum and subsequently through a 5 μ m and 0.45 μ m syringe filter. The filtrate was concentrated by using Ultrafree-15 Units
15 (Millipore, exclusion weight 30 kDa) followed by three washing steps with 20 mM Tris HCl (pH 8.2). After centrifugation at 10.000 g for 5 min the supernatant of the concentrate (20 – 60 fold) was applied to a Source Q15 column ((10mm, length 40 mm) equilibrated with 20 mM Tris HCl, pH 8.2. Proteins were eluted by a linear gradient from 0 to 800 mM NaCl over 50
20 ml at a flow rate of 2 ml/min (Fig. 1A). The purity of the fractions was determined by SDS-PAGE and subsequent rapid silver staining. APIT appears as a band at 60 kDa. Cytolytic activity was measured as APIT-induced reduction of the metabolic activity of Jurkat cells via turnover of WST (see example 2). Enzymatic activity was determined as described
25 in example 3. Fractions which show high purity and cytotoxic respectively enzymatic activity (Fig. 1A; fraction 42 to 48) were pooled, concentrated and loaded onto a Superose 12 HR 10/30 column (Pharmacia). Proteins were eluted with 100 mM potassium phosphate buffer (pH 7.2) at a flow rate of 0.5 ml/min The first peak represents the active APIT (Fig. 2B;
30 fraction 11 to 14).

Example 2: Phenotype of APIT-induced cell death

The purple fluid of *Aplysia punctata* contains a cytolytic activity which induces rapid and extensive death of Jurkat T cells in culture. APIT induces cell death of tumor cells which resembles neither apoptosis nor necrosis. In order to classify the APIT-induced cell death we looked for common features of apoptosis and necrosis.

Jurkat T cells were harvested in the log phase, centrifuged and adjusted to a density of 5×10^5 /ml with fresh medium (RPMI supplemented with 10% FCS, 100 U/ml penicillin and 100 μ g/ml streptomycin). Cells were cultured with APIT, cycloheximide as a positive control or medium at 37°C, 5% CO₂ and 100% humidity for the indicated times. Fragmented DNA of apoptotic cells was analyzed according to Herrmann et al. (1994, Nucleic Acid Research 22: 5506-5507). Cell vitality was determined as metabolic activity via the turnover of WST-1 (ROCHE, Mannheim) to red formazan by the mitochondrial dehydrogenase of viable cells. Absorbance of the cell suspension was measured photometrically at 450 nm (690 nm reference). Toxicity was measured by quantifying propidium iodide uptake (1 μ g/ml in PBS) by Flow Cytometry.

Morphologically, tumor cells treated with ink or APIT did not exhibit typical morphological apoptotic or necrotic signs of cell death (Fig. 2A), and neither blebbing nor swollen cells were detected when cells were treated with a lethal dose of ink. Cells did not form clusters anymore, cytoplasm became translucent and nuclei prominent (Fig. 2A). The intracellular movements of plasma and organelles stopped, detachment and formation of vacuoles were observed when adherent cells were incubated with APIT (data not shown). Consistent with the absence of apoptosis, fragmented DNA or nuclei were not detected in ink-treated tumor cells (Fig. 2 B); moreover, caspases were not activated (data not shown). Metabolic activity of tumor cells was blocked as early as 30 min after exposure to ink

or APIT (Fig. 2C). Ink-treated tumor cells rapidly took up propidium iodide (PI) indicating plasma membrane permeabilization and cell death (Fig. 2D).

Example 3: Stability of APIT

APIT was further characterized by its sensitivity to heat, low pH and high concentrations of urea.

For determination of its heat sensitivity native ink was dialyzed against PBS at 4°C for several days to separate chromopeptides. Dialysed ink was incubated for 10 min at the indicated temperatures, and activity was measured immediately as enzymatic production of H₂O₂. This assay is based on the finding that APIT transforms L-lysine to H₂O₂ and α -keto acid. The production of H₂O₂ was determined via the turnover of ABTS (2,2-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) to a green formazan in the presence of H₂O₂ by horseradish peroxidase. Heat-treated ink was incubated with L-lysine (1 mM) in 100 μ l 100 mM potassium phosphate buffer, pH 7.2 for 10 min at 25°C. The reaction was stopped by adding 1 μ l of 10 M phosphoric acid. To 25 μ l of this solution 1 mM ABTS and 1 Unit horseradish peroxidase was added in 225 μ l 100 mM potassium phosphate buffer, pH 5.0. Absorption was measured photometrically at 405 nm (reference 690 nm).

Purified APIT was challenged to different pH-values by adding a mixture of monobasic and dibasic potassium phosphate and phosphoric acid rendering the desired pH. After a 10 min incubation pH of samples was adjusted to pH 7.2 by adding appropriate amounts of dibasic phosphate. Afterwards enzymatic activity was measured as H₂O₂-production as described above.

The activity of APIT after treatment with urea was measured via the production of α -keto acid, which was quantified photometrically by its

reaction with the hydrazone MBTH (3-methyl-2-benzothiazolone hydrazone hydrochloride) as described by Soda (1968). Dialyzed ink was incubated with urea at indicated concentrations for 30 min. Subsequently the remaining enzymatic activity was measured without removing urea for 15 min at 25°C. As control, defined amounts of α -keto isocaproic acid (Sigma; K-0629) were treated equally.

APIT was characterized by its heat sensitivity and was found to exhibit a high and constant activity after pre-incubation for 10 min at 0°C to 50°C. Activity was clearly reduced at 60°C and absent at temperatures of 70°C or higher (Fig. 3A). APIT also shows a loss of activity with decreasing pH, with complete inactivation after a 10 min pre-incubation at pH 3 or lower (Fig. 3B). An outstanding feature of APIT is its resistance to urea (Fig. 3C). After 30 min treatment with 6 M urea, the activity of APIT was almost unaffected. At 8 M urea, the activity was reduced by about 50%.

Example 4: Sequencing and cloning of APIT

In order to clone the cDNA of APIT N-terminal and internal peptide sequences were identified by PMF (peptide mass fingerprint), ESI/MS and Edman degradation (Fig. 4A). A suitable internal peptide sequence was used to design a degenerated primer for PCR (Fig. 4A, underlined sequence) with reverse transcribed mRNA, prepared from *Aplysia punctata* tissues. Subsequent 5'-RACE yielded the full length cDNA which was cloned and analyzed.

Amino acid sequencing by peptide mass fingerprint (PMF), ESI/MS and Edman degradation. Purified APIT was separated by SDS PAGE and 2 DE gel electrophoresis (Thiede et al., 2001, J. Biol. Chem. 276: 26044-26050). The N-terminus of APIT was identified from a single band/spot of a PVDF blot by Edmann degradation. For the identification of internal peptide sequences a single band/spot was punched from the gel, digested

with trypsin and dissolved in aqueous trifluoroacetic acid (Thiede et al., 2001, J. Biol. Chem. 276:26044-26050). Tryptic peptides were separated using a Smart-HPLC system with a column of 2.1 mm inner diameter and 10 cm length (μ RPC C2/C18 SC 2.1/10, Smart System, Pharmacia Biotech, Freiburg, Germany) and an acetonitrile gradient in 0.1% (v/v) trifluoroacetic acid at a flow rate of 100 μ l/min at room temperature. The peptide fractions were dried, dissolved in 6 μ l 0.3% (v/v) aqueous trifluoroacetic acid/acetonitrile (2:1) and analyzed by MALDI-MS. The mass spectra were recorded by using a time-of-flight delayed extraction MALDI mass spectrometer (Voyager-Elite, Perseptive Biosystems, Framingham, MA, USA) as previously described (Thiede et al., 2001, J. Biol. Chem. 276:26044-26050). Briefly, fifty mg/ml 2,5-dihydroxybenzoic acid in 0.3% (v/v) aqueous trifluoroacetic acid/acetonitrile (2:1) was used as matrix and 0.3 μ l of the sample and 0.3 μ l of the matrix were mixed and applied to a gold-plated sample holder and introduced into the mass spectrometer after drying. The spectra were obtained in the reflectron mode by summing 50-150 laser shots. For N-terminal sequencing peptide fractions containing single masses were loaded onto a Biobrene-coated glass fiber filter, transferred to a PVDF membrane and excised. Sequencing was performed using a Procise sequencer (Applied Biosystems, Weiterstadt, Germany).

Cloning of the APIT gene. In order to dissect mantle gland, nidadamental gland, digestive gland and opaline gland some animals were relaxed by injection of 5 – 10 ml sterile $MgCl_2$ solution (380 mM). Isolated tissues were frozen immediately in liquid nitrogen. Total RNA was prepared from these tissues using the „peq gold TRIfast“ reagent (Peqlab). mRNA was reverse transcribed using the tagged oligo dT oligonucleotide 5'-tcc taa cgt agg tct aga cct gtt gca $t_{(18)}$ -3' (Fig. 4B, oligo 1) and the Superscript II polymerase (LIFE) at 42°C. In order to amplify a fragment of the APIT gene the degenerated primer 5'-tc gtg ttc gar tac tci gay cg-3' derived from the APIT peptide VFEYSDR (Fig. 4B, oligo 2) and the specific primer 5'- ctg tag gtc tag acc tgt tgc a-3' (Fig. 4B, oligo 3) directed against the tag sequence

of the oligo dT-primer was used. PCR was performed with the „expand long template“ system (ROCHE, Mannheim) at 68 °C and the product was cloned into the pCMV-vector (Stratgene) and sequenced. The 5' terminal cDNA of APIT was cloned using the 5' RACE System (LIFE) according to the manufacturers instructions. Primers 5'-ccg tgt aga tct cac tgc cat a-3'

(Fig. 4B, oligo 4) or 5'-ccg ttg agt tgt aga cct-3 (Fig. 4B, oligo 6) were combined with the primers 5'-ggc cac gcg tcg act agt acg ggi igg gii ggg iig-3' (Fig. 4B, oligo 5) or 5'-aatt ggc cac gcg tcg act agt ac-3' (Fig. 4B, oligo 7) to yield a product which was cloned into the pCDNA3-vector (Invitrogen) and sequenced. Finally, full length APIT cDNA was obtained by amplifying the APIT using the specific primers 5' – aa ttc tcg tct gct gtg ctt ctc ct (Fig. 4B, oligo 8) and 5' – gac tta gag gaa gta gtc gtt ga (Fig. 4B, oligo 9) and cloned into the pGEX-4T3 Vector (Amersham). DNA from 3 clones of transfected E.coli was prepared and sequenced.

The identity of the isolated gene was confirmed by comparing the computed translational product (Fig. 4C) with the amino acid sequences of the tryptic peptides (Fig. 4A) and the peptide mass fingerprint. It consisted of 1608 bp coding for a protein of 535 amino acids (Fig. 4C) with the predicted mass of 60,167 dalton and a pI of 4.59. The N-terminal 18 amino acids of APIT comprised a putative secretion signal sequence which was absent from the mature protein, most likely due to posttranslational modification during secretion. Furthermore, APIT exhibited homology to FAD-binding oxidoreductases with a conserved dinucleotide binding fold around amino acids 39 to 66 followed by a so-called GG-motif typical for certain oxidases like LAAO, MAO. (Fig. 4C). (Dailey et al., 1998, J.Biol. Chem. 273:13658-13662; Vallon et al., 2000, Proteins 38:95-114; Macheroux et al., 2001 Eur. J. Biochem. 268:1679-1686). The highest degree of homology existed to the Cyplasin from *A. punctata*, the Aplysianin from *A. kurodai* and the mucus-toxin of the giant African snail *Achatina fulica*.

Comparing the 3 derived DNA-sequences we often found differences in the third position of coding triplets which nevertheless only seldom produced changes in the amino acid sequence of APIT (Fig. 4C).

5 By the method described above, further 11 clones were isolated from *Aplysia punctata* which have a homology to the sequences described in Fig. 4 of at least 95%. Several mutations of the amino acid sequence were found in the domain comprising the dinucleotide binding fold and the GG motif, which probably have no effect upon the function (Fig. 4D). In Pos.
10 22 of SEQ ID NO: 2, C is replaced by S in two clones. In Pos. 52, A is replaced by T in one clone. In Pos. 60, L is replaced by Q in 7 clones. In Pos. 69, D is replaced by H in one clone. In Pos. 77, T is replaced by S in one clone.

15 **Example 5: FAD association**

The toxic and enzymatic activity of APIT is due to the presence of an attached FAD.

20 In order to purify the tumor lytic activity, ink from *A. punctata* was subjected to different purification protocols and afterwards each fraction was tested for its toxic activity (see example 1). Activity always correlated with the presence of a protein of approximately 60 kDa (Fig. 5 A and B). Moreover, APIT was found to contain carbohydrate residues using the DIG
25 Glycan/Protein double labeling method (Roche; data not shown). Furthermore, all spectra of the highly active fractions exhibited a double peak at 390/470 nm (Fig. 5C) which is characteristic for protein bound flavines (Massey et al., 2000, Biochem Soc. Trans. 28:283-96). Heating of
30 APIT for 10 min to 60°C, which is accompanied by a substantial loss of activity also results in loss of detectable FAD-absorption, as is the case with lowering the pH to inactivating values around pH 3. Heating and

pH-challenge of APIT was performed as described in example 3 (data not shown).

Consistently, APIT contained the conserved dinucleotide binding fold
5 involved in pyrophosphate binding (Wierenga et al., 1986, J. Mol. Biol.,
187:101-107) which is found in many flavoproteins (Fig. 4B; example 4).
Moreover, in APIT like in many oxidases a so-called GG-motif is found
adjacent to the dinucleotide binding fold (Dailey et al., 1998, J. Biol.
Chem. 273:13658-13662, Vallon et al., 2000, Proteins, 38:95-114).
10 Based on the structure of the dinucleotide binding fold and conserved
sequence motifs, FAD containing proteins are ordered into 4 families (Dym
et al., 2001, Protein Sci. 10:1712-28). According to this classification and
based on homology APIT belongs to the Glutathione reductase 2 family
(GR2) (Dym et al., 2001, Protein Sci. 10:1712-28). The data show that
15 FAD is a necessary prosthetic group for toxic and enzymatic activity of
APIT.

Example 6: Cell-death is mediated via H_2O_2

20 Proteome analysis revealed that thioredoxin peroxidase II is involved in the
APIT mediated tumor cell death. Thioredoxinperoxidase II is involved in
detoxification of reactive oxygen species (ROS) by reducing hydrogen
peroxides as well as other peroxides. We therefore tested whether H_2O_2 is
produced during APIT incubation and found that H_2O_2 is the mediator of
25 APIT-induced cell death. Scavenging this toxic compound by catalase
results in survival of APIT treated cells.

H_2O_2 production was measured after incubation of APIT in medium alone
and in cell suspension as described in example 3. Toxicity was measured
30 by quantifying propidium iodide uptake ($1 \mu\text{g/ml}$ in PBS) by Flow
Cytometry. Cell vitality was determined as metabolic activity via the
turnover of WST-1 (ROCHE, Mannheim) to red formazan by the

mitochondrial dehydrogenase of viable cells. Absorbance of the cell suspension was measured photometrically at 450 nm (690 nm reference).

As shown in Fig. 6A, APIT induced the production of H_2O_2 in the presence (167 μM) as well as in absence of cells (280 μM). This strongly argues for an enzymatic activity of APIT which transforms medium ingredients under the production of hydrogen peroxide. In the presence of cells the measured H_2O_2 amount is somewhat lower which might be explained by cellular consumption and degradation of H_2O_2 . In the absence of APIT H_2O_2 was not detectable. To investigate whether the APIT-induced cell death is mediated by H_2O_2 , cells were treated with APIT in the presence of the H_2O_2 degrading enzyme catalase and then stained with PI. Catalase completely abolished the ink-induced increase of PI stained cells (Fig. 6B). Degradation of H_2O_2 by catalase also inhibited the rapid break-down of metabolic activity induced by APIT (Fig. 6C) but, as expected, was ineffective in blocking CD95 (Fas/Apo-1)-induced cell death in the same assay (Fig. 6C). In the presence of catalase APIT no longer induced morphological changes of tumor cells as judged by microscopic investigation (Fig. 6D). The highly efficient inhibition by catalase in particular suggested that no other substance than H_2O_2 elicits the toxic effect observed in APIT-treated samples. Consistently, H_2O_2 induced the phenotype typical for APIT-treated cells (Fig. 6D). Furthermore, proteome analyses revealed changes in H_2O_2 treated cells which were characteristic of APIT-treated cells. These data together clearly demonstrated that the cytotoxic activity depended on the H_2O_2 producing enzymatic activity of APIT.

Example 7: APIT is a L-lysine/L-arginine a-oxidase. Enzymatic activity is a prerequisite for toxicity

APIT produced H_2O_2 in RPMI medium in the absence of cells. In order to identify the substrates in cell culture medium which are converted to H_2O_2 by APIT, we prepared different media with defined amino acid composition

by supplementing HEPES buffered modified Krebs Ringer medium (KRG: 25 mM HEPES pH 7.4, 125 mM NaCl, 5 mM KCl, 1.2 mM KH_2PO_4 , 5 mM NaHCO_3 , 6 mM glucose, 1.2 mM MgSO_4 , 1 mM CaCl_2) with 10% FCS, 2 mM glutamine, essential and non-essential amino acids (Invitrogen), or
5 single essential amino acids in concentrations equivalent to RPMI medium (Invitrogen). Media were adjusted to pH 7.4 and filter sterilized. After incubation of these media with purified APIT the enzymatic activity was measured as H_2O_2 production via turnover of ABTS (2,2-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) to a green formazan in the presence
10 of H_2O_2 and horseradish peroxidase (Fig. 7A and Table 1).

In a next step we checked whether the substrate specificity could be impaired by digest of APIT. For proteolytic digest aliquots of dialysed ink were treated for 2 h with proteinase K (0,05 mg/ml final) in PBS at 37°C.
15 Reaction was stopped by adding aprotinin (1 $\mu\text{g}/\text{ml}$ final) or PEFA ([4-(2-aminoethyl)-benzolsulfonyl fluoride-hydrochloride]-hydrochloride; 0,25 mg/ml final), and digest was checked on a 15% SDS-PAGE. After incubation of digested ink with different amino acid compositions in potassium phosphate buffer the enzymatic activity was measured as H_2O_2
20 production (Fig. 7B).

In order to test whether withdrawal of L-lysine and L-arginine results in rescue of APIT-treated cells we incubated Jurkat cells in medium lacking L-lysine and L-arginine. Control cells were cultured in a medium containing
25 L-lysine(HCl (40 mg/l) and L-arginine(HCl (240 mg/l). Toxicity was measured by quantifying propidium iodide uptake (1 $\mu\text{g}/\text{ml}$ in PBS) by Flow Cytometry (Fig. 7C).

Cell vitality was determined as metabolic activity via the turnover of
30 WST-1 (ROCHE, Mannheim) to red formazan by the mitochondrial dehydrogenase of viable cells. Absorbance of the cell suspension was

measured photometrically at 450 nm (690 nm reference). As control tumor cells were killed by anti-CD95 treatment (Fig. 7D).

5 α -Keto acids were quantified photometrically by their reaction with the hydrazone MBTH (3-methyl-2-benzothiazolone hydrazone hydrochloride) as described (Soda et al., 1968, Anal. Biochem. 25:228-235) (Fig. 7E).

10 The K_m value for L-lysine was determined as H_2O_2 production and calculated according to Michaelis Menten with the GraphPad Prism 3.0 software (GraphPad Software, San Diego California USA) using non linear regression (Fig. 7F).

15 Surprisingly, from all amino acids tested only L-lysine and L-arginine served as substrates for APIT to produce hydrogen peroxide (Fig. 7A). Moreover, the restricted substrate specificity was even maintained when APIT was digested with protease K suggesting that the protease resistant fragment of APIT contains both, the active domain and the domain which determines the substrate specificity (Fig. 7B). These data were confirmed by functional analyses which showed that APIT was unable to induce cell death (Fig. 7C) or reduce metabolic activity (Fig. 7D) in tumor cells incubated in medium lacking L-lysine and L-arginine, indicating that the enzymatic activity of APIT is the prerequisite for its toxicity. L-lysine and L-arginine deprivation had no influence on the metabolic activity of tumor cells under the experimental conditions (Fig. 7D). Activation of CD95(Fas/Apo-1) efficiently impaired cell vitality irrespective of the presence of L-lysine or L-arginine (Fig. 7D), demonstrating that cell death can be induced under L-lysine and L-arginine limited conditions.

30 As shown in the reaction scheme in figure 7G, α -keto derivatives are produced by amino acid oxidases and these could indeed be demonstrated when L-lysine was used as substrate for APIT (Fig. 7E). These results suggested that APIT catalyses the formation of H_2O_2 by the reaction

outlined in figure 7G. Kinetic studies analyzed according to Michaelis-Menten revealed a K_m of 0.182 mM for L-lysine (Fig. 7F).

By adding L-lysine (2-50 μ g/ml) to tumor cells which are cultured with APIT (20 ng/ml) in medium depleted of L-lysine and L-arginine or in pure FCS, the metabolic activity of the tumor cells can be reduced down to 16% respectively 50% of the control cells without additional L-lysine. This shows that the tumorolytic effect of APIT can be manipulated by changing the amount of available substrate which is of significance for *in vivo* studies and/or for application of APIT in pharmaceutical compositions and/or methods for treatment of cancer.

Example 8: Sensitivity of different tumor cell lines to APIT induced cell death.

Tumor cells were harvested in the log phase. Triplicates of each 50,000 cells were cultured in a flat bottomed 96-well-plate in 100 μ l medium with increasing concentrations of APIT. After 14 hours the metabolic activity of the cells was determined by addition of 10 μ l WST-1 per well (ROCHE, Mannheim). The yellow tetrazolium salt is cleaved to red formazan by cellular enzymes of viable cells. The metabolic activity correlates with cell vitality and was quantified by measuring the absorbance of the dye solution with a spectrophotometer at 450 nm (reference 650 nm).

APIT is able to kill different tumor cells. T and B cell leukemia cell lines (Jurkat neo, CEM neo, SKW neo), a chronic myelogenous leukemia cell line (K562), and cells from an orphan and aggressive osteosarcoma (Ewings tumor: RDES, A673) showed the highest sensitivity to the APIT induced cell death ($IC_{50} \leq 5.6$ ng/ml), followed by cells derived from small cell lung cancer (GLC4, GLC4/ADR), cervix cancer (Chang) and acute monocytic leukemia (THP-1) ($IC_{50} \leq 10$ ng/ml). Most of the adherent growing cells of solid tumors (breast cancer: MCF-7, SK-BR-3; prostate

cancer: PC3, DU-145; colon cancer: HT-29; cervix cancer: HeLa; uterus cancer: Hec-1-B; larynx cancer HEP-2; stomach cancer: AGS; liver cancer: Hep G2) and the monocyte leukemia cell line MonoMac 6 are less sensitive at the indicated cell concentration ($IC_{50} \leq 20$ ng/ml), but become more sensitive when lower cell concentrations were used (IC_{50} 5 - 10 ng/ml).

Resistance to apoptosis as well as multi drug resistance (MDR) represent severe problems in cancer therapy. It is therefore of particular interest that APIT kills apoptosis resistant cell lines as well as MDR cancer cell lines equally efficient as their non-resistant counter parts (Tab. 2): Over-expression of apoptosis inhibitors of the Bcl-2 family in acute lymphoblastic leukemia cell lines (CEM Bcl-X_L, Jurkat Bcl-2) as well as in B cell leukemia (SKW Bcl-2) (Tab. 2; 4th row) does not protect from APIT mediated cell death and results in IC_{50} values of ≤ 6 ng/ml, similar to the non-transfected parental cell lines, confirming that APIT induce cell death in an apoptosis independent way. The MDR cell line GLC4/ADR (Tab. 2, 5th row) was generated by selection with doxorubicin (Zijlstra et al., 1987, Cancer Res. 47:1780-1784). Its multifactorial MDR is caused by over-expression of MRP-1 and a decreased activity of the DNA topoisomerase II. GLC4/ADR cells possess almost the same sensitivity to APIT (IC_{50} 10 ng/ml) as the parental line GLC4 does (IC_{50} 9 ng/ml).

Example 9: Proteome analysis: change in protein expression pattern in Jurkat T cells after treatment with APIT

Treatment with APIT. Jurkat T cells (5×10^5 /ml) were incubated with APIT (20 ng/ml) for 8 h at 37 °C in 5.0% CO₂ in the presence of 1 µg/ml cycloheximide. Controls were performed without APIT.

Total cell lysate. The Jurkat T cells were solubilized in 5 volumes of a buffer containing 9 M urea, 25 mM Tris/HCl, pH 7.1, 50 mM KCl, 3 mM EDTA, 70 mM DTT, 2.9 mM benzamidine, 2.1 µM leupeptin, 0.1 µM

pepstatin, 1 mM PMSF, and 2% carrier ampholytes (Servalyte pH 2-4, Serva, Heidelberg, Germany). After 30 minutes of gentle stirring at room temperature, the samples were centrifuged at 100000 g (Ultracentrifuge Optima TLX, Beckman, München, Germany) for 30 minutes with a
5 TLA120.2 rotor, which were kept at room temperature before centrifugation. The clear supernatant was frozen at -70°C.

Proteomics. The methods of preparing 2-DE gels, staining with Coomassie Blue G-250, staining with silver nitrate, in-gel tryptic digestion, peptide
10 mass fingerprinting by MALDI-MS, and identification of the proteins are described in Jungblut et al., Molecular Microbiology, 2000, 36, 710-725.

Identification was performed using the peptide mass fingerprinting analysis software MS-Fit (<http://prospector.ucsf.edu/ucsfhtml3.2/msfit.htm>) or
15 ProFound (<http://canada.proteometrics.com/prowl-cgi/ProFound.exe?FORM=1>). Searches were performed in the databases NCBI nr and SwissProt. The proteins are referenced by the genbank identifier, accession number and/or version number.

20 **Results.** APIT induces either upregulation, downregulation, or modification of the proteins. Modification in the context of this example is a change in the apparent mass and/or the apparent pI value of the protein. By comparison of 2-DE patterns of APIT-treated whole cell lysates with the corresponding patterns of untreated cells, the proteins as described in
25 Table 3 were identified to be affected by APIT.

Example 10: Transcriptome analysis

The influence of APIT on the gene expression of tumor cells was
30 investigated by Microarray technology.

***In situ* Oligonucleotide Arrays.** A custom oligonucleotide glass array of specific 60mer oligonucleotides representing the mRNA of about 8500 human genes was designed based on human Unigene clusters (Unigene build No. 148) including positive and negative control oligonucleotides (*Homo sapiens* house keeping genes and *Arabidopsis thaliana* genes respectively). The probe design included a base composition filter and a homology search to minimise cross-hybridisation.

RNA isolation, labelling and hybridisation to arrays. Jurkat neo cells (1×10^7 in 20 ml) were cultured for 2 hours in medium (RPMI + 10 % FCS) in the presence or absence of APIT (10 ng/ml) at 37°C, 5% CO₂. Cells were harvested and the pellet was dissolved in 2 ml Trizol (Life Technologies). Total RNA was extracted after addition of chloroform and subsequent centrifugation and precipitated with isopropanol. After washing the pellet with 75% ethanol it was briefly air-dried. Quality control of the RNA included exclusion of genomic DNA by PCR and "Lab on a chip technology" (Bioanalyser). RNA (5 µg) from each pool was amplified using a reverse transcriptase/T7 polymerase. 1.5 µg of test cRNAs labelled either with Cy3 or Cy5 were hybridised for 16 hours at 65°C to arrays. Each sample was also labelled and hybridised with the reverse fluorophore to obviate possible dye bias. Slides were scanned using a Microarray scanner. Background signal was determined using negative control spots and subtracted, data were normalised relative to non-regulated genes. Data from duplicate hybridizations were combined.

Results. Tables 4 and 5 summarize the genes with increased or decreased transcription rate of treated cells compared with untreated cells, indicating these genes and/or its gene products (proteins) to be targets of APIT and/or H₂O₂.

Example 11: Knock down of Prx I sensitized tumor cells for APIT induced cell death.

Peroxiredoxin I (Prx I) exhibited the most significant modification observed in 2-DE protein patterns of APIT treated cells in comparison to untreated Jurkat cells (Table 3). The modification of Prx I which is observed in 2-DE gel analysis of APIT treated cells resembles that described for the oxidized and inactivated Prx I, indicating that APIT inactivates this detoxification system. In order to investigate the role of Prx I for the APIT induced cell death we performed knock down of Prx I expression by RNA interference (RNAi). If Prx I was involved in the detoxification of H_2O_2 produced by APIT, we expected to observe a sensitization in cells in which Prx I expression is decreased.

Therefore, 20.000 HeLa cells/well were seeded in a 96 well plate one day prior to transfection. Transfection was performed with 0.25 μ g siRNA directed against

Prx I having the sequence (SEQ ID NO: 9):

5' -GGCUGAUGAAGGCAUCUCGdTdT-3'
3' -dTdTCCGACUACUCCGUAGAGC-5',

Lamin A/C having the sequence (SEQ ID NO: 30):

5' -CUGGACUCCAGAAGAACAdTdT
3' -dTdTGACCUGAAGGUCUUCUUGU-5',

and Luciferase having the sequence (SEQ ID NO: 31):

5' -CUUACGCUGAGUACUUCGAdTdT-3'
3' -dTdTGA AUGCGACUCAUGAAGCU-5',

as control and 2 μ l transmessenger per well using the transmessenger transfection kit (Qiagen, Hilden, Germany) according to manufacturers instructions. For APIT treatment (40ng/ml) transfections were conducted in triplicates. 24 h after transfection cells were splitted and grown for additional 48 h before fresh medium with or without APIT was added for 6h. Assay conditions which led to a 50 to 70 % reduction of the metabolic activity of treated cells were chosen for RNAi experiments. Metabolic activity was determined as described in Example 2. In parallel, RNA from

about 50.000 cells was isolated using the RNeasy 96 BioRobot 8000 system (Qiagen) 48 h after transfection. The relative amount of mRNA was determined by realtime PCR using Quantitect™ SYBR Green RT-PCR Kit from Qiagen following manufacturers instructions. The expression level of Prx mRNA was normalised against the internal standard GAPDH. The following primers were used: Prx I 5': CTGTTATGCCAGATGGTCAG, Prx I 3': GATACCAAAGGAATGTTCATG, Lamin A/C 5':CAAGAAGGAGGGTGACCTGA, Lamin A/C 3':GCATCTCATCCTGAAGTTGCTT, GAPDH 5':GGTATCGTGGAAGGACTCATGAC, GAPDH 3':ATGCCAGTGAGCTTCCCGTTCAG.

To measure sensitization, conditions were chosen under which the reduction of metabolic activity of treated cells was 50 % or less of the untreated cells. siRNAs were transfected into HeLa cells and after 72 h cells were treated with APIT for 6 h and metabolic activity was determined. In parallel, cells were harvested for quantitative analysis of the respective mRNAs by realtime PCR (Fig. 8 A). The mRNA of Prx I was reduced by more than 90 % compared to the mRNA level of GAPDH. Interestingly, this reduction of Prx I expression significantly sensitized the cells for killing by APIT whereas control siRNA directed against Luciferase and Lamin A/C had no effect (Fig. 8 B). Our data show that knock down of Prx I by RNAi rendered the cells hypersensitive for APIT suggesting that Prx I is part of an H₂O₂ detoxifying pathway which is modulated by APIT.

In summary, we identified the modification of Prx I, as an important step in the APIT of this detoxification system. The fact that the knock down of Prx I expression by RNAi increased the sensitivity of tumor cells for the cytolytic activity of APIT underlines the impact of Prx 1 RNA interference for cancer therapy.

Example 12: APIT does not induce actin depolymerisation

Cyplasin S and L, proteins from *Aplysia punctata* which induce cell death of tumor cells were described to cause fast actin depolymerisation in human tumor cells (see WO 03/057726). The influence of APIT treatment on actin filaments by fluorescence staining of actin by Phalloidin-TRITC (Tetramethylrhodamin- isothiocyanat) is investigated.

HeLa cells (1.5×10^5 cells/well/ml) were cultured over night on cover slips in 12 well plates. Subsequently, cells were incubated in the presence or absence of APIT (40 ng/ml) for 6 h or Cytochalasin D ($1 \mu\text{M}$; Sigma 8273) for 30 min. After washing in PBS, cells on cover slips were fixed for 10 min in 3,7 % PFA (paraformaldehyde), washed again and finally permeabilized by a 1 min incubation in 0,5 % Triton X-100. Blocking of unspecific binding sites by incubation in PBS, 1% FCS, 0,05 % Tween 20 was followed by actin staining with Phalloidin-TRITC in blocking puffer for 15 min and 3 fold washing. Nuclei were stained by the presence of Hoechst 33258 in the last washing step. Cover slips were investigated by fluorescence microscopy.

As shown in Fig. 9 untreated cells (A) possess a typical actin cytoskeleton. Incubation in the presence of Cytochalasin (B), an inducer of rapid actin depolymerisation, resulted in a massive loss of actin filaments and an accumulation of actin in clumps. In contrast, APIT(C) did not induce actin depolymerisation in HeLa cells. APIT treated cells remain their actin filaments, even after 6 h when the plasma membrane was already disrupted (see example 2, Fig. 2D). This clearly differentiates APIT induced cell death from that induced by Cyplasins.

Example 13: Healthy human cells are resistant against the APIT-induced cell death

To analyze the specificity of APIT for tumor cells, normal human umbilical vein endothelial cells (HUVEC) and tumor cells (Jurkat cells) were

incubated with increasing amounts of purified APIT and analyzed for lactate dehydrogenase (LDH) release (Fig. 10).

5 HUVEC and Jurkat cells (50,000 cells/100 μ l/wells) were treated with increasing amounts of APIT in a 96 well plate. After over night incubation half of the culture supernatants (50 μ l) were transferred in fresh wells and mixed with 50 μ l reagent of Cytotoxicity Detection Kit-LDH according to the manufacturers instruction (Roche 1644793). Release of LDH in the supernatant is found only, when cells were killed by APIT. LDH release
10 was calculated as the ratio of LDH activity of APIT treated cells relative to the LDH activity of Triton X 100 lysed cells.

Jurkat cells showed a dramatic release of LDH upon incubation with APIT (Fig. 10). In contrast, even at the highest APIT concentrations used in this
15 experiments (40 ng/ml), APIT treated HUVEC cells only showed a minor LDH release below 10 %, indicating a strong resistance of these normal cells against the cytolytic activity of APIT. As several tumor cell lines showed a similar APIT sensitivity as the Jurkat cells (Table 2), the data suggest the toxic effect induced by APIT is tumor specific.



Claims

1. A polypeptide comprising the amino acid sequence shown in SEQ ID
5 NO: 2, 4, or 6.
2. A polypeptide claimed in Claim 1 which is an oxidase which is
capable to produce H_2O_2 .
- 10 3. A polypeptide as claimed in any one of the Claims 1 to 2 which is
an alpha amino acid oxidase.
4. A polypeptide as claimed in Claim 3 which is a L-lysine and/or L
arginine oxidase.
- 15 5. A polypeptide comprising a fragment of the polypeptide as claimed
in any one of the Claims 1 to 4.
6. A polypeptide as claimed in Claim 5 which is obtained by protease
20 digestion of the polypeptide as claimed in any of the Claims 1 to 4.
7. A polypeptide as claimed in Claim 6 which is obtained by proteinase
K digestion.
- 25 8. A polypeptide as claimed in Claim 5 comprising the sequence
selected from amino acid residue No. 39 to 77 in SEQ ID NO: 2.
- 30 9. A polypeptide as claimed in Claim 8 comprising 1 to 20 additional
amino acid residues at the N-terminus and/or the C-terminus
selected from the sequences of SEQ ID NO: 2 or SEQ ID NO: 4
adjacent to the sequence selected in claim 8.

10. A polypeptide as claimed in Claim 8 comprising 1 to 10 additional amino acid residues at the N-terminus and/or the C-terminus selected from the sequences of SEQ ID NO: 2 adjacent to the sequence selected in claim 8.

5

11. A polypeptide as claimed in Claim 8 comprising 1 to 5 additional amino acid residues at the N-terminus and/or the C-terminus selected from the sequences of SEQ ID NO: 2 adjacent to the sequence selected in claim 8.

10

12. A polypeptide as claimed in any one of the Claims 2 to 11, wherein the H_2O_2 producing activity can be regulated by the addition or removal of an L-amino acid.

15

13. A polypeptide as claimed in Claim 12 which is regulated by L-lysine, L-arginine, a derivative or precursor of L-lysine, a derivative or a precursor of L-arginine, or a mixture thereof.

20

14. A polypeptide which has an identity to the polypeptides of any of the claims 1 to 13 of at least 70%.

15. A polypeptide as claimed in any one of the claims 1 to 14 which is a recombinant polypeptide.

25

16. The polypeptide as claimed in claim 15, which is a fusion polypeptide.

17. A nucleic acid encoding a polypeptide of any of the Claims 1 to 16.

30

18. The nucleic acid of Claim 17 comprising
- (a) a nucleotide sequence as shown in SEQ ID NO: 1, 3, or 5, or at least the polypeptide coding portion thereof, or the complement thereof, or
 - 5 (b) a nucleotide sequence corresponding to the sequence of (a) within the scope of degeneracy of the genetic code, or the complement thereof, or
 - (c) a nucleotide sequence hybridizing under stringent condition with the sequence of (a) and/or (b), or
 - 10 (d) a nucleotide sequence which has a homology of at least 70% to the sequences of (a) and/or (b).

19. The nucleic acid of claim 17 or 18 operatively linked to an expression control sequence.

20. The nucleic acid of any one of claims 17 to 19 which is a recombinant vector.

21. A recombinant cell comprising the nucleic acid of any one of the Claims 17 to 20.

22. An antibody directed against a polypeptide of any one of the Claims 1 to 16.

23. A pharmaceutical composition or a kit of pharmaceutical compositions comprising the polypeptide as claimed in any of the Claims 1 to 16, in a pharmaceutically effective amount and optionally together with suitable diluents, carriers and/or adjuvants.

24. The pharmaceutical composition or kit of Claim 23 comprising at least one further component which is a substance capable of modulating the cytotoxic activity of the polypeptide.

25. The pharmaceutical composition or kit of Claim 24, wherein the polypeptide and the modulating substances are provided as separate preparations.

5 26. The pharmaceutical composition or kit of Claim 25, wherein the polypeptide is provided for administration before the modulating substances.

10 27. The pharmaceutical composition or kit of any one of the Claims 24 to 26, wherein the modulating substance selected from (i) L-lysine, L-arginine, a derivative or precursor of L-lysine, a derivative or precursor of L-arginine, or a mixture thereof, and/or (ii) a flavine nucleoside.

15 28. The pharmaceutical composition or kit of any one of the Claims 24 to 27, further comprising a nucleic acid, and/or a recombinant cell, and/or an APIT inhibitor.

20 29. The pharmaceutical composition or kit of Claim 28, wherein the inhibitor is an antibody against the polypeptide.

30. A polypeptide, and/or a nucleic acid, and/or a recombinant cell, and/or an inhibitor as claimed in any one of the Claims 1 to 22, for use in a diagnostic or therapeutic method in humans or animals.

25 31. A polypeptide, and/or a nucleic acid, and/or a recombinant cell, and/or an inhibitor as claimed in Claim 30 for diagnosis or treatment of cancer.

- 5 32. A polypeptide, and/or a nucleic acid, and/or a recombinant cell, and/or an inhibitor as claimed in Claim 30 or 31 for diagnosis or treatment of lung cancer, breast cancer, prostate cancer, colon cancer, cervix cancer, uterus cancer, larynx cancer, stomach cancer, liver cancer, Ewings sarkoma, acute lymphoid leukemia, chronic myeloid leukemia, apoptosis resistant leukemia, MDR lung cancer, pancreas cancer, gastric cancer, kidney cancer, gliomas, melanomas, chronic lymphoid leukemia, and/or lymphoma.
- 10 33. Use of a substance as described in Table 3 or/and Table 4 or/and Table 5 as target substance for a polypeptide of any one of Claim 1-16.
- 15 34. Use of claim 33 in which the target substance is a protein.
35. Use of claim 34 in which the target substance is a peroxidase, particularly peroxiredoxin I.
- 20 36. Use of claim 35 in which the target substance comprises
(a) the amino acid sequence shown in SEQ ID NO: 8, or/and
(b) an amino acid sequence which is homologous to the sequence of (a) with at least 70%, or/and
(c) a fragment of the amino acid sequence of (a) or (b).
- 25 37. Use of claim 33 in which the target substance is a nucleic acid.
38. Use of claim 37 in which the target substance codes for a peroxidase, particularly peroxiredoxin I.

39. Use of claim 38 in which the target substance comprises
- (a) the nucleotide sequence shown in SEQ ID NO: 7, or/and
 - (b) a nucleotide sequence which corresponds to the sequence of (a) within the scope of the degeneracy of the genetic code, or/and

5

- (c) a nucleotide sequence hybridizing to the sequence of (a) or/and (b) under stringent conditions, or/and
- (d) a fragment of the nucleotide sequence of (a), (b) or (c).

- 10 40. Use of a substance of any one of the claims 33 to 39 for the identification of new pharmaceutical agents, particularly in a screening method.

- 15 41. A pharmaceutical composition or kit comprising as an active agent a combination of APIT and at least one inhibitor of a substance of any one of claims 33 to 39.

- 20 42. An inhibitor of peroxiredoxin I activity which is an RNA molecule, particularly a double stranded RNA molecule comprising a nucleic acid of at least 15 nucleotides complementary to a peroxiredoxin I transcript.

- 25 43. An inhibitor as claimed in claim 42, wherein the peroxiredoxin I transcript is derived from the sequence of SEQ ID NO: 7.

- 30 44. An inhibitor as claimed in claims 42 or 43, wherein the one or two strands independently have a length of 19 to 25 nucleotides, preferably 19 to 23 nucleotides.

45. An inhibitor as claimed in any of the claims 42 to 44 which is a double-stranded RNA molecule having a sequence selected from the group of sequences consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11; SEQ ID NO: 12; SEQ ID NO: 13; SEQ ID NO: 14; SEQ ID NO: 15; SEQ ID NO: 16; SEQ ID NO: 17; SEQ ID NO: 18; SEQ ID NO: 19; SEQ ID NO: 20; SEQ ID NO: 21; SEQ ID NO: 22; SEQ ID NO: 23; SEQ ID NO: 24; SEQ ID NO: 25; SEQ ID NO: 26; SEQ ID NO: 27; SEQ ID NO: 28; SEQ ID NO: 29, optionally with one or two 3' overhangs and optionally one or more modified nucleotides.

46. A pharmaceutical composition or kit comprising an inhibitor or a nucleic acid encoding an inhibitor as claimed in any one of the claims 42 to 45.

47. A pharmaceutical composition as claimed in claim 46, comprising a gene therapy delivery system suitable for the delivery of a nucleic acid encoding the inhibitor as claimed in any of the claims 42 to 45 to predetermined tissues or/and cell types.

48. Use of an inhibitor as claimed in any of the claims 42 to 45 for the manufacture of a medicament for the diagnosis or/and treatment of cancer.

49. A pharmaceutical composition or kit comprising

(i) a polypeptide obtainable from *Aplysia*, comprising an amino acid sequence selected from:

(a) D-G-E-D-A-A-V (SEQ ID NO:32) and/or

(b) (D/Q)-G-(I/V)-C-R-N-(Q/R)-R-(Q/P) (SEQ ID NO:33),

(c) F-A-D-S (SEQ ID NO:34),

(d) G-P-D-G-(I/L)-V-A-D (SEQ ID NO:35),

(e) P-G-E-V-S-(K/Q)-(I/L) (SEQ ID NO: 36),

- (f) A-T-Q-A-Y-A-A-V-R-P-I-P-A-S-K (SEQ ID NO:37),
- (g) D-S-G-L-D-I-A-V-E-Y-S-D-R (SEQ ID NO:38),
- (h) G-D-V-P-Y-D-L-S-P-E-E-K (SEQ ID NO: 39) or/and
- (i) SEQ ID NO: 41, 43, 44, 45.

5

or a fragment thereof,

wherein the polypeptide or fragment has cytotoxic activity,
or/and a nucleic acid comprising

- (i) a nucleotide sequence as shown in SEQ ID NO: 40 or
42 or at least the polypeptide coding portion thereof or
the complement thereof,
- (ii) a nucleotide sequence corresponding to the sequence
of (i) within the scope of degeneracy of the genetic
code, or the complement thereof, or/and
- (iii) a nucleotide sequence hybridizing under stringent
conditions with the sequence of (i) or/and (ii), and

10

15

- (II) an inhibitor of a target substance as described in Table 3
or/and Table 4 or/and Table 5.

50. A method for the diagnosis or treatment of cancer, wherein the
pharmaceutical composition or kit as claimed in claims 41, 46, 47 or
49 is administered to a subject in need thereof.

20

19 Nov. 2003

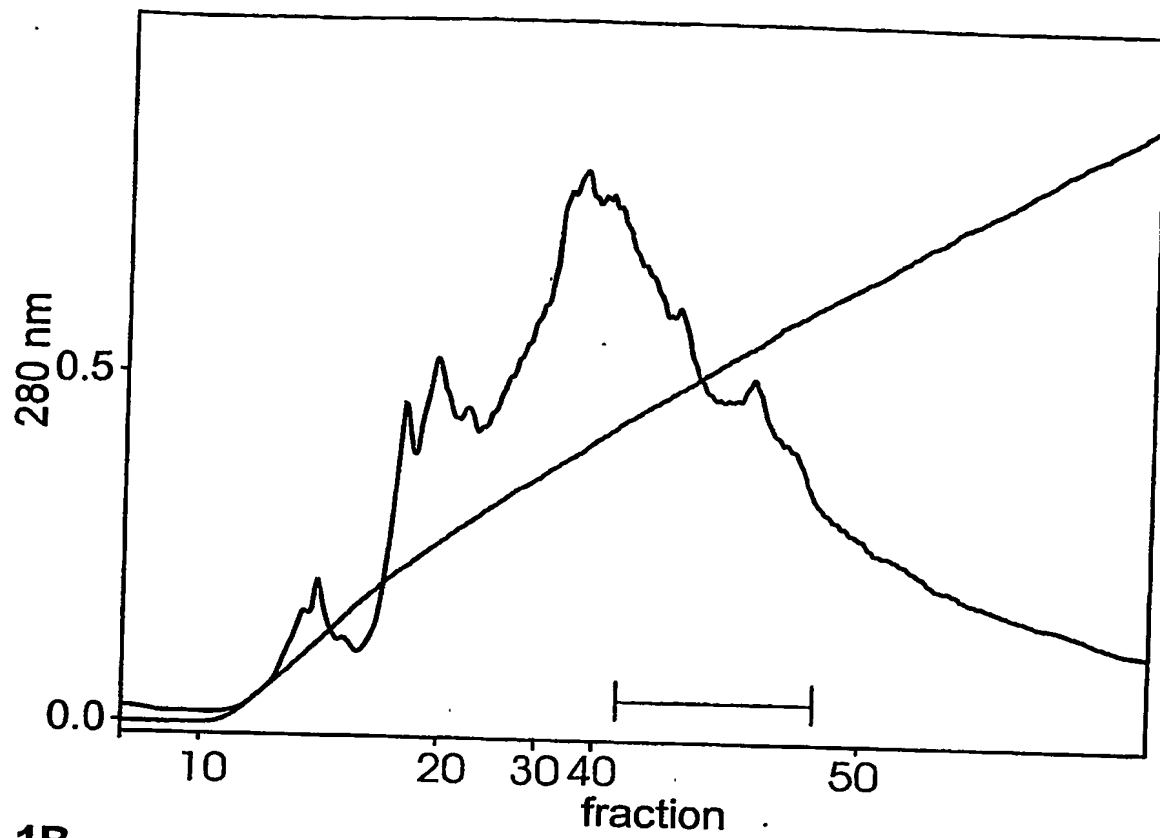
Abstract

5 The present invention relates to a cytotoxic polypeptide which is an
L-amino acid oxidase isolated from the ink of the sea hare *Aplysia
punctata*.

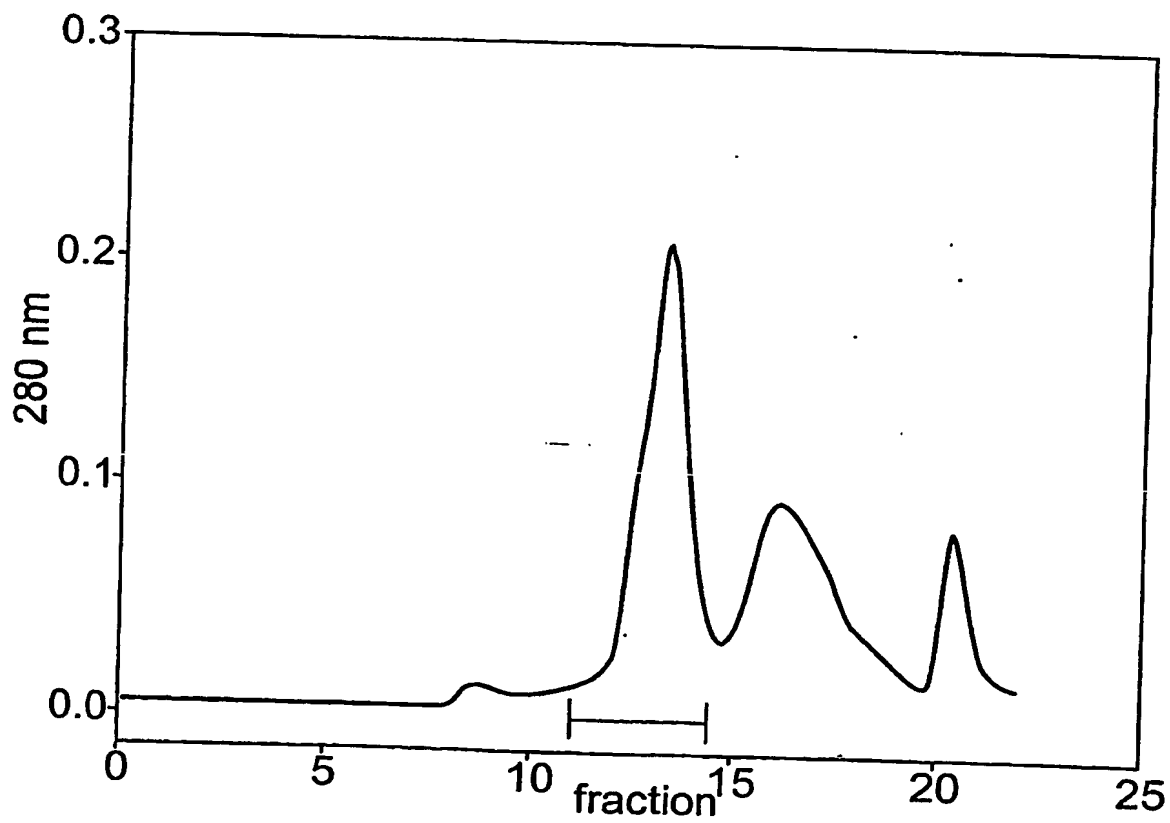
10 kt 18.11.03

19. Nov. 2003

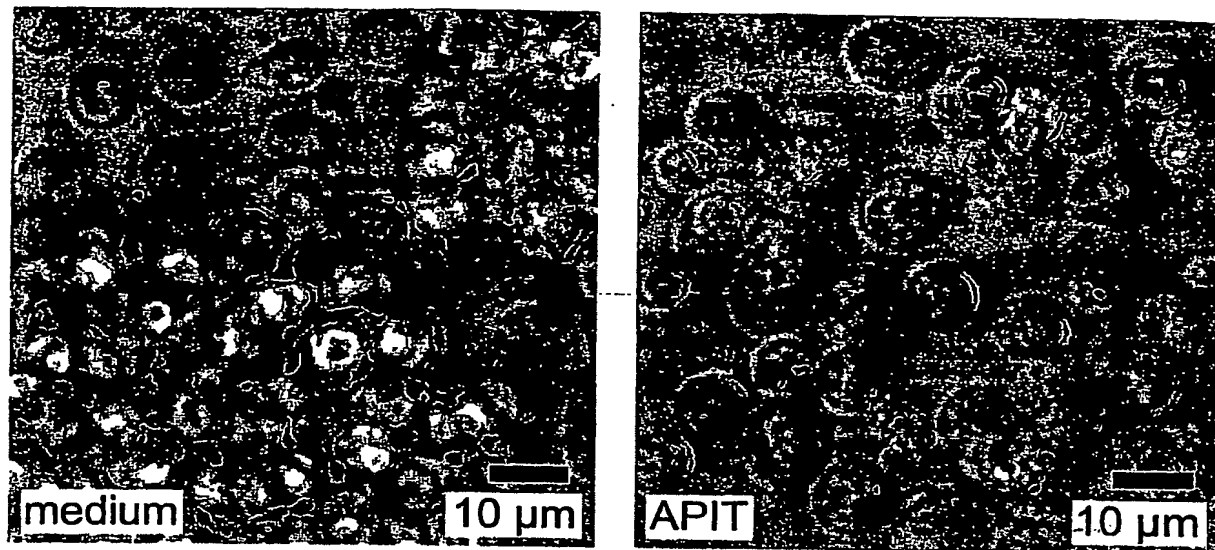
1A



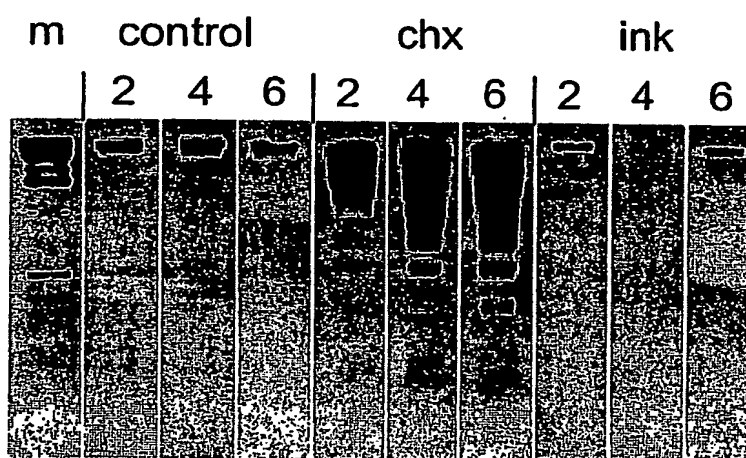
1B



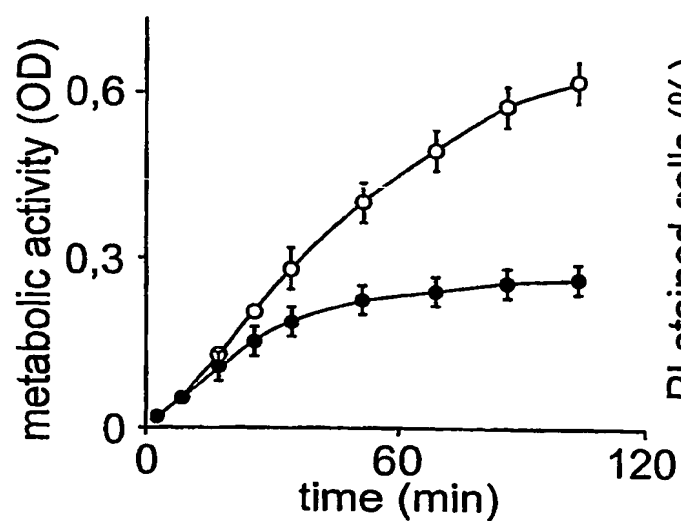
2A



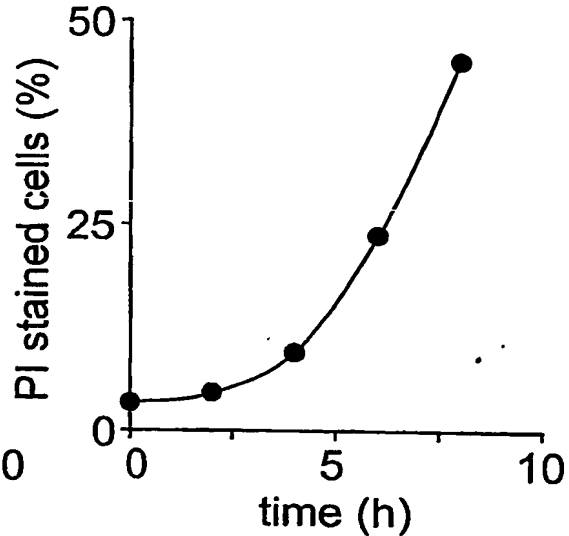
2B

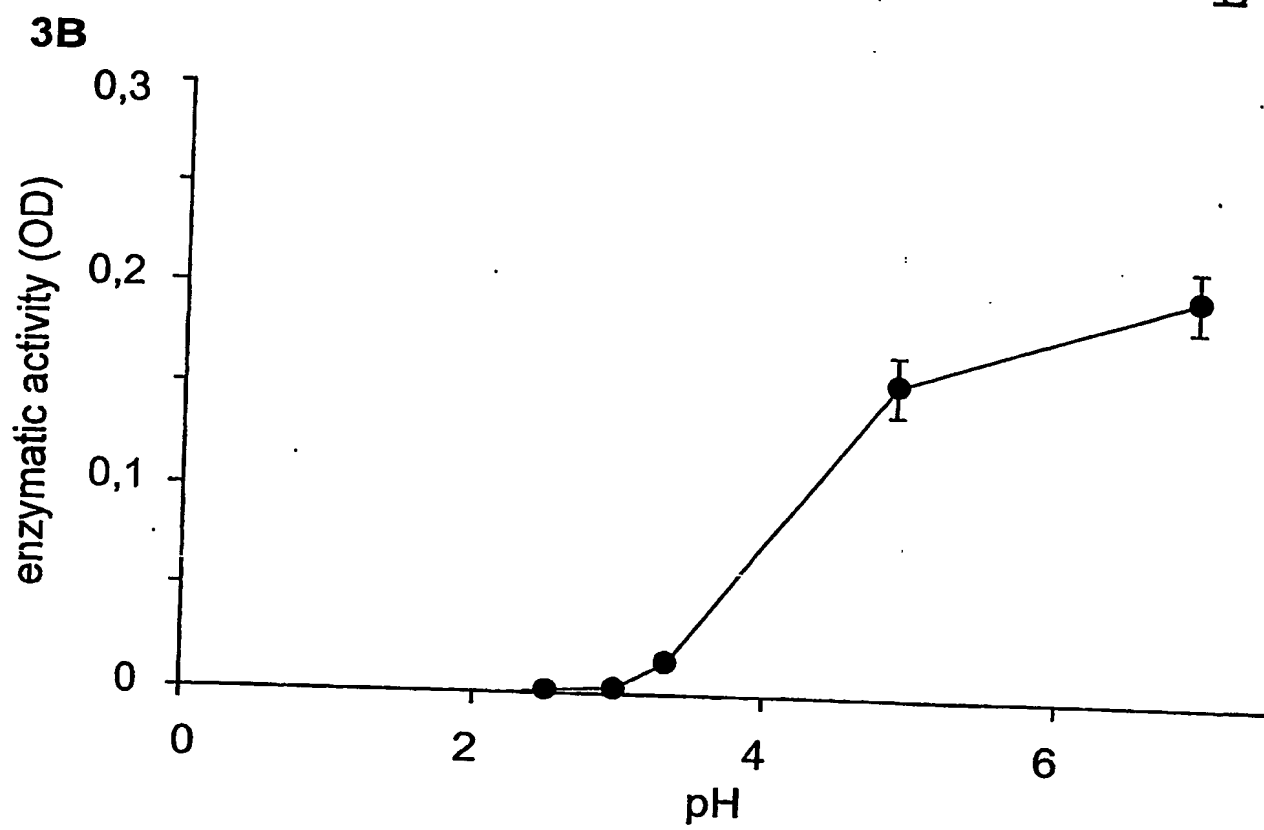
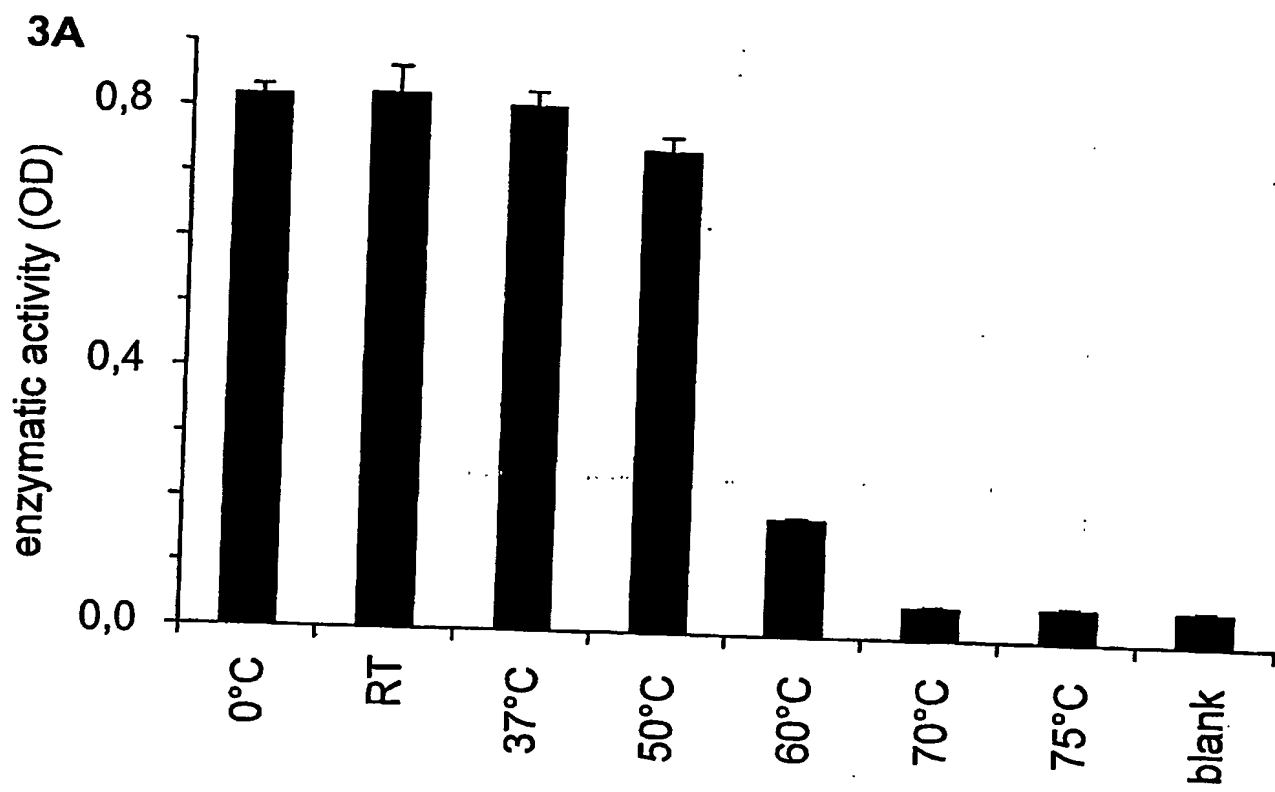


2C



2D





3C

extinction (320nm)

0,6
0,4
0,2
0

0

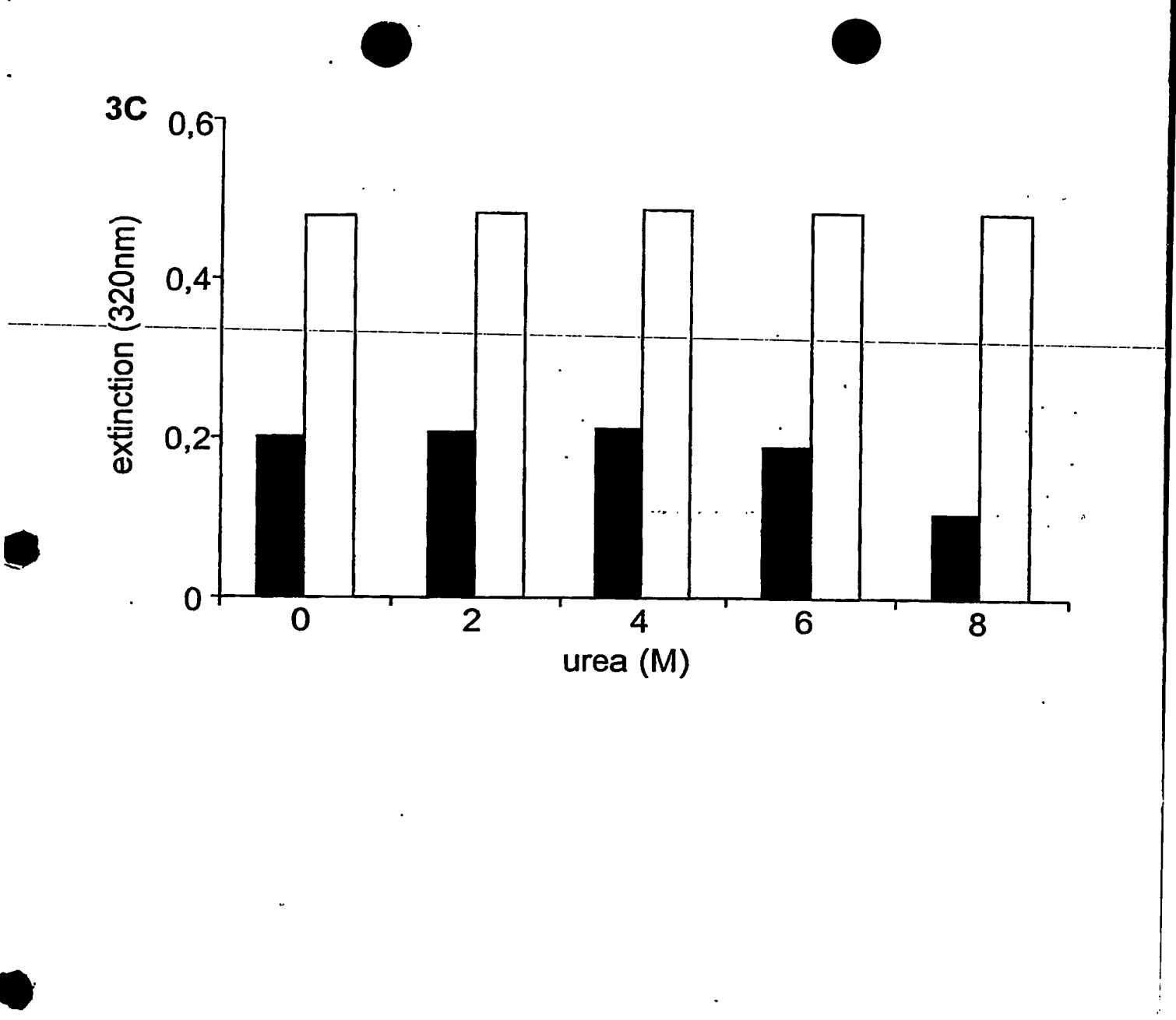
2

4

6

8

urea (M)



4A

N-terminal sequence:

D-G-I-C-R-N-Q-R-Q
Q V R P

Internal peptide sequences

	Sequence
1	DSGLDIAVFEYSDR
2	LFXYQLPNTPDVNLEI
3	VISELGLTPK
4	XGDVPYDLSPEEK
5	VILAXPVYALN
6	ATQAYAAVRPIPASK
7	VFMTFDQP
8	SDALFFQMYD
9	SEASGDYILIASYADGLK
10	NQGEDIPGSDPQYNQVTEP(L)(K)

X = not determinable

underlined: primer sequence for RT-PCR

4B

1	Oligo-dT DBuTag1	tcc taa cgt agg tct aga cct gtt gca ttt ttt ttt ttt ttt ttt
2	V-Fey 3 DTS 5'	tc gtg ttc gar tac tci gay cg
3	DBuTag1 DTS 3'	ctg tag gtc tag acc tgt tgc a
4	ATF Race 3' 660	ccg tgt aga tct cac tgc cat a
5	Abrided Anchor Primer	ggc cac gcg tcg act agt acg ggi igg gii ggg iig
6	ATF Race 3' 436	ccg ttg agt tgt aga cct
7	AUAP-EcoRI	aatt ggc cac gcg tcg act agt ac
8	ATF 5' Sign Eco RI GEX/ET	aa ttc tcg tct gct gtg ctt ctc ct
9	ATF 3' XhoI	gac tta gag gaa gta gtc gtt ga

4C

M S S A V L L L A C A L V I S V H A D G IV C
ATGTCGTCTGCTGTGCTTCTCCTGGCTTGTGCGTTGGTCATCTCTGTCCACGCCGACGGTATCTGC
...TCGTCTGCTGTGCTTCTCCTGGCTTGTGCGTTGGTCATCTCTGTCCACGCCGACGGTGTCTGC
.....GACGGTATCTGC

R N R R Q C N R E V C G S T Y D V A V V G A
AGAAACAGACGTCAATGTAACAGAGAGGTGTGCGGTTCTACCTACGATGTGGCCGTCGTGGGGGCG
AGAAACAGACGTCAATGTAACAGAGAGGTGTGCGGTTCTACCTACGATGTGGCCGTCGTGGGGGCG
AGAAACAGACGTCAATGTAACAGAGAGGTGTGCGGTTCTACCTACGATGTGGC GTCTGTGGGGGCG

G P G G A N S A Y M L R D S G L D I A V F E
GGGCCTGGGGGAGCTAACTCCGCCTACATGCTGAGGGACTCCGGCCTGGACATCGCTGTGTTTCGAG
GGGCCTGGGGGAGCTAACTCCGCCTACATGCTGAGGGACTCCGGCCTGGACATCGCTGTGTTTCGAG
GGGCCTGGGGGAGCTAACTCCGCCTACATGCTGAGGGACTCCGGCCTGGACATCGCTGTGTTTCGAG

Y S D R V G G R L F T Y O L P N T P D V N L
TACTCGACCGAGTGGGCGGCCGGCTGTTACCTACCAGCTGCCCAACACACCCGACGTAACTCT
TACTCAGACCGAGTGGGCGGCCGGCTGTTACCTACCAGCTGCCCAACACACCCGACGTAACTCT
TACTCAGACCGAGTGGGCGGCCGGCTGTTACCTACCAGCTGCCCAACACACCCGACGTAACTCT

E I G G M R F I E G A M H R L W R V I S E L
GAGATTGGCGGCATGAGGTTTCATCGAAGGCGCCATGCACAGGCTCTGGAGGGTCATTTTCAGAACTC
GAGATTGGCGGCATGAGGTTTCATCGAGGGCGCCATGCACAGGCTCTGGAGGGTCATTTTCAGAACTC
GAGATTGGCGGCATGAGGTTTCATCGAGGGCGCCATGCACAGGCTCTGGAGGGTCATTTTCAGAACTC

G L T P K V F K E G F G K E G R Q R F Y L R
GGCCTAACCCCAAGGTGTTCAAGGAAGGTTTCGGCAAGGAGGGCAGACAAGATTTTACCTGCGG
GGCCTAACCCCAAGGTGTTCAAGGAAGGTTTCGGAAAGGAGGGCAGACAGAGATTTTACCTGCGG
GGCCTAACCCCAAGGTGTTCAAGGAAGGTTTCGGAAAGGAGGGCAGACAGAGATTTTACCTGCGG

G Q S L T K K Q V K S G D V P Y D L S P E E
GGACAGAGCCTGACCAAGAAACAGGTCAAGAGTGGGGACGTACCCTATGACCTCAGCCCGGAGGAG
GGACAGAGCCTGACCAAGAAACAGGTCAAGAGTGGGGACGTACCCTATGACCTCAGCCCGGAGGAG
GGACAGAGCCTGACCAAGAAACAGGTCAAGAGTGGGGACGTACCCTATGACCTCAGCCCGGAGGAG

K E N Q G N L V E Y Y L E K L T G L OK L N G
AAAGAAAACCAGGGAAATCTGGTCAATACTACCTGGAGAACTGACAGGTCTACAACCTCAACGGC
AAAGAAAACCAGGGAAATCTGGTCAATACTACCTGGAGAACTGACAGGTCTACAACCTCAA TGGT
AAAGAAAACCAGGGAAATCTGGTCAATACTACCTGGAGAACTGACAGGTCTA AACTCAACGGC

EG P L K R E V A L K L T V P D G R F L Y D L
GACCGCTCAAACGTGAGGTTGCGCTTAACTAACCCTGCCGGACGGCAGATTCTCTATGACCTC
GAACCGCTCAAACGTGAGGTTGCGCTTAACTAACCCTGCCGGACGGCAGATTCTCTATGACCTC
GACCGCTCAAACGTGAGGTTGCGCTTAACTAACCCTGCCGGACGGCAGATTCTCTATGACCTC

S F D E A M D L V A S P E G K E F T R D T H
TCGTTTGACGAAGCCATGGATCTGGTTGCCTCCCCTGAGGGCAAAGAGTTACCCGAGACACGCAC
TCGTTTGACGAAGCCATGGATCTGGTTGCCTCCCCTGAGGGCAAAGAGTTACCCGAGACACGCAC
TCGTTTGACGAAGCCATGGA CTGGTTGCCTCCCCTGAGGGCAAAGAGTTACCCGAGACACGCAC

4C (continued)

V F T G E V T L DG A S A V S L F D D H L G E
 GTCTTCACAGGAGAGGTCACCCTGGACGCGTCGGCTGTCTCCCTCTTCGACGACCACCTGGGAGAG
 GTCTTCACCGGAGAGGTCACCCTGGGCGCGTCGGCTGTCTCCCTCTTCGACGACCACCTGGGAGAG
 GTGTTACCGGAGAAGTCACCCTGGACGCGTCGGCTGTCTCCCTCTTCGACGACCACCTGGGAGAG

D Y Y G S E I Y T L K E G L S S V P Q G L L
 GACTACTATGGCAGTGAGATCTACACCCTAAAGGAAGGACTGTCTTCCGTCCCACAAGGGCTCCTA
 GACTACTACGGCAGTGAGATCTACACCCTCAAGGAAGGACTGTCTTCCGTCCCCAAGGGCTCCTA
 GACTACTATGGCAGTGAGATCTACACCCTAAAGGAAGGACTGTCTTCCGTCCCACAAGGGCTCCTA

Q AT F L D A A D S N E F Y P N S H L K A L R
 CAGGCTTTTCTGGACGCCGCAGACTCCAACGAGTTCTATCCCAACAGCCACCTGAAGGCCCTGAGA
 CAGGCTTTTCTGGACGCCGCAGACTCCAACGAGTTCTATCCCAACAGCCACCTGAAGGCCCTGAGA
 CAGACTTTTCTGGACGCCGCAGACTCCAACGAGTTCTATCCCAACAGCCACCTGAAGGCCCTGAGA

R K T N G Q Y V L Y F E P T T S K D G Q T T
 CGTAAGACCAACGGTCAGTATGTTCTTTACTTTGAGCCCACCACCTCCAAGGATGGACAAACCACA
 CGTAAGACCAACGGTCAGTATGTTCTTTACTTTGAGCCCACCACCTCCAAGGATGGACAAACCACA
 CGTAAGACCAACGGTCAGTATGTTCTTTACTTTGAGCCCACCACCTCCAAGGATGGACAAACCACA

I N Y L E P L Q V V C A Q R V I L A M P V Y
 ATCAACTATCTGGAACCCCTGCAGGTTGTGTGTGCACAAAGAGTCATCCTGGCCATGCCGGTATAC
 ATCAACTATCTGGAACCCCTGCAGGTTGTGTGTGCACAGAGAGTCATTCTGGCCATGCCGGTCTAC
 ATCAACTATCTGGAACCCCTGCAGGTTGTGTGTGCACAGAGAGTCATCCTGGCCATGCCGGTCTAC

A L N Q L D W N Q L R N D R A T O A Y A A V
 GCTCTGAACCAACTAGACTGGAATCAGCTCAGAAATGACCGAGCCACCCAAGCGTACGCTGCCGTT
 GCTCTCAACCAGTGGATTGGAATCAGCTCAGAAATGACCGAGCCACCCAAGCGTACGCTGCCGTG
 GCTCTCAACCAACTGGACTGGAATCAGCTCAGAAATGACCGAGCCACCCAAGCGTACGCTGCCGTG

R P I P A S K V F M TS F D O P W W L E N E R
 CGCCCGATTCTGCAAGTAAGGTGTTTCATGTCCTTTGATCAGCCCTGGTGGTTGGAGAACGAGAGG
 CGCCCGATTCTGCAAGTAAGGTGTTTCATGACCTTTGATCAGCCCTGGTGGTTGGAGAACGAGAGG
 CGCCCGATTCTGCAAGTAAAGTGTTTCATGACCTTTGATCAGCCCTGGTGGTTGGAGAACGAGAGG

K S W V T K S D A L F S O M Y D W Q K S E A
 AAATCCTGGGTCACCAAGTCGGACGCGCTTTTCAGCCAAATGTACGACTGGCAGAAGTCTGAGGCG
 AAATCCTGGGTCACCAAGTCGGACGCGCTTTTCAGTCAAATGTACGACTGGCAGAAGTCTGAGGCG
 AAATCCTGGGTCACCAAGTCGGACGCGCTTTTCAGCCAAATGTACGACTGGCAGAAGTCTGAGGCG

S G D Y I L I A S Y A D G L K A Q Y L R E L
 TCCGGAGACTACATCCTGATCGCCAGCTACGCCGACGGCCTCAAAGCCCAGTACCTGCGGGAGCTG
 TCCGGAGACTACATCCTGATCGCCAGCTACGCCGACGGCCTCAAAGCCCAGTACCTGCGGGAGCTG
 TCCGGAGACTACATCCTGATCGCCAGCTACGCCGACGGCCTCAAAGCCCAGTACCTGCGGGAGCTG

K N O G E D I P G S D P G Y N O V T E P L K
 AAGAATCAGGGAGAGGACATCCCAGGCTCTGACCCAGGCTACAACCAGGTTACCGAACCCCTCAAG
 AAGAATCAGGGAGAGGACATCCCAGGCTCTGACCCAGGCTACAACCAGGTACCGAACCCCTCAAG
 AAGAATCAGGGAGAGGACATCCCAGGCTCTGACCCAGGCTACAACCAGGTACCGAACCCCTCAAG

4C (continued)

D T I L D H L T E A Y G V E R D S I PR E P V
 GACACCATTCTTGACCACCTCACTGAGGCTTATGGCGTGGAGCGAGACTCGATCCCGGAACCCGTG
 GACACCATTCTTGACCACCTCACTGAGGCTATGGCGTGGAGCGAGACTCGATCCCGGAACCCGTG
 GACACCATTCTTGACCACCTCACTGAGGCTTATGGCGTGGAGCGAGACTCGATCCCGGAACCCGTG

T A A S Q F W T D Y P F G C G W I T W R A G
 ACCGCCGCTTCCCAGTTCTGGACAGACTACCCGTTTGGCTGTGGATGGATCACCTGGAGGGCCGGC
 ACCGCCGCTTCCCAGTTCTGGACAGACTACCCGTTTGGCTGTGGATGGATCACCTGGAGGGCCGGC
 ACCGCCGCTTCCCAGTTCTGGACGACTACCCGTTGGCTGTGGATGGATCACCTGGAGGGCCGGC

F H F D D V I S T M R R P S L K D E V Y V V
 TTCCATTTTCGATGACGTCATCAGCACCATGCGTCGCCCGTCACTGAAAGATGAGGTATACGTGGTG
 TTCCATTTTCGATGACGTCATCAGCACCATGCGTCGCCCGTCACTGAAAGATGAGGTCTACGTGGTG
 TTCCATTTTCGATGACGTCATCAGCACCATGCGTCGCCCGTCACTGAAAGATGAGGTCTACGTGGTG

G A D Y S W G L I S S W I E G A L E T S E N
 GGAGCCGACTACTCCTGGGGACTTATCTCCTCCTGGATAGAGGGCGCTCTGGAGACCTCGGAAAAC
 GGAGCCGACTACTCCTGGGGACTTATCTCCTCCTGGATAGAGGGCGCTCTGGAGACCTCGGAAAAC
 GGAGCCGACTACTCCTGGGGACTTATCTCCTCCTGGATAGAGGGCGCTCTGGAGACCTCGGAAAAC

V I N D Y F L -
 GTCATCAACGACTACTTCCTCTAA
 GTCATCAACGACTACTTCCTCTAA
 GTCATCAACGACTACTTCCTCTAA

4D

VS

MSSAVLLLLACALVISVHADGICRNRRCNREVCGSTYDVAVVGA

10

20

30

40

T

Q

H

S

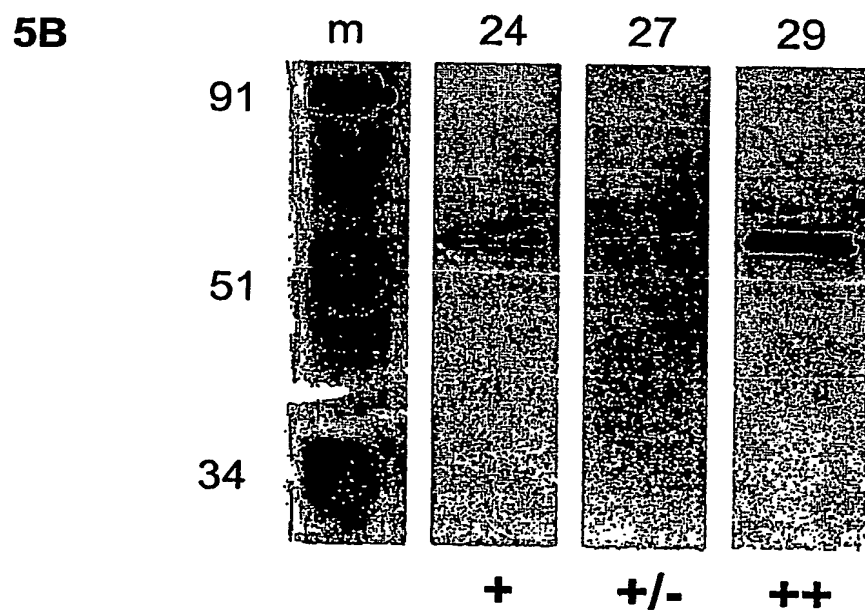
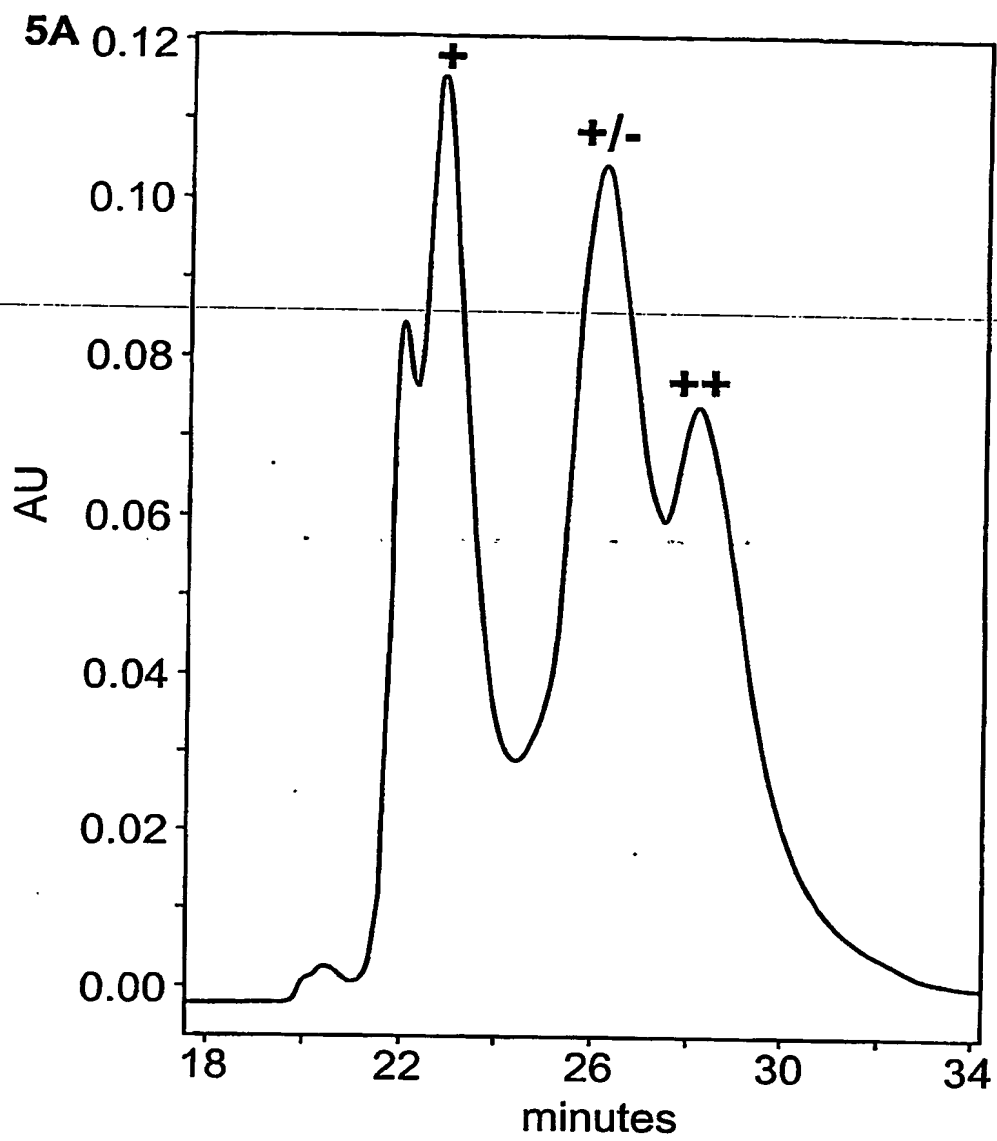
GPGGANSAYMLRDSGLDIAVFEYSDRVGGRLFTYQLPNTPDVNL

50

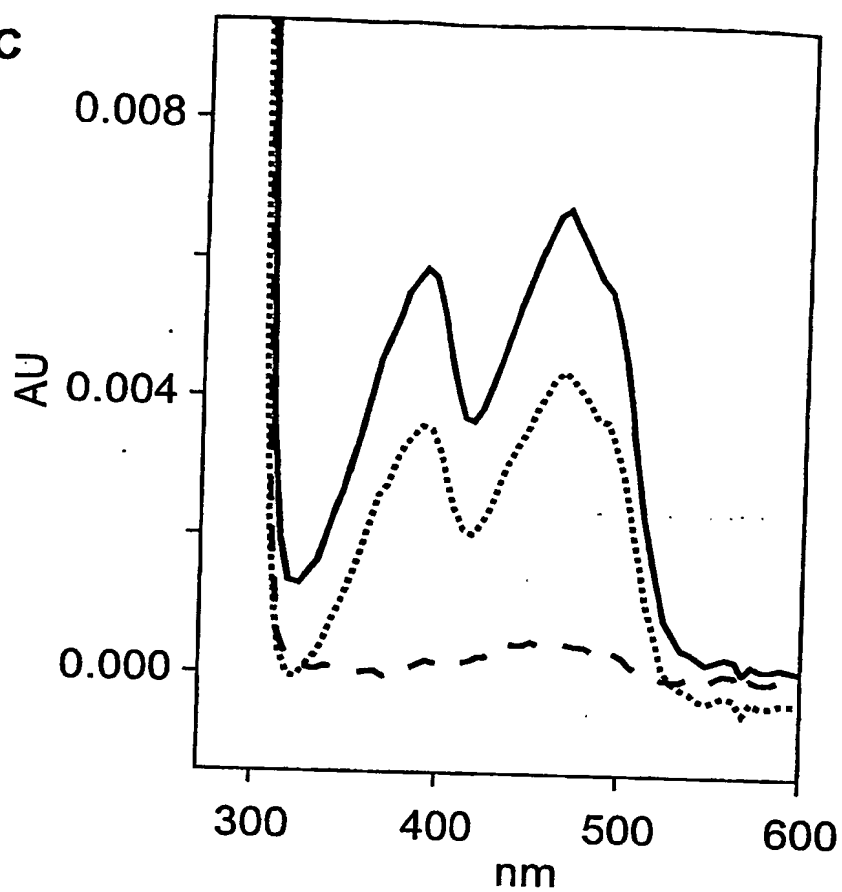
60

70

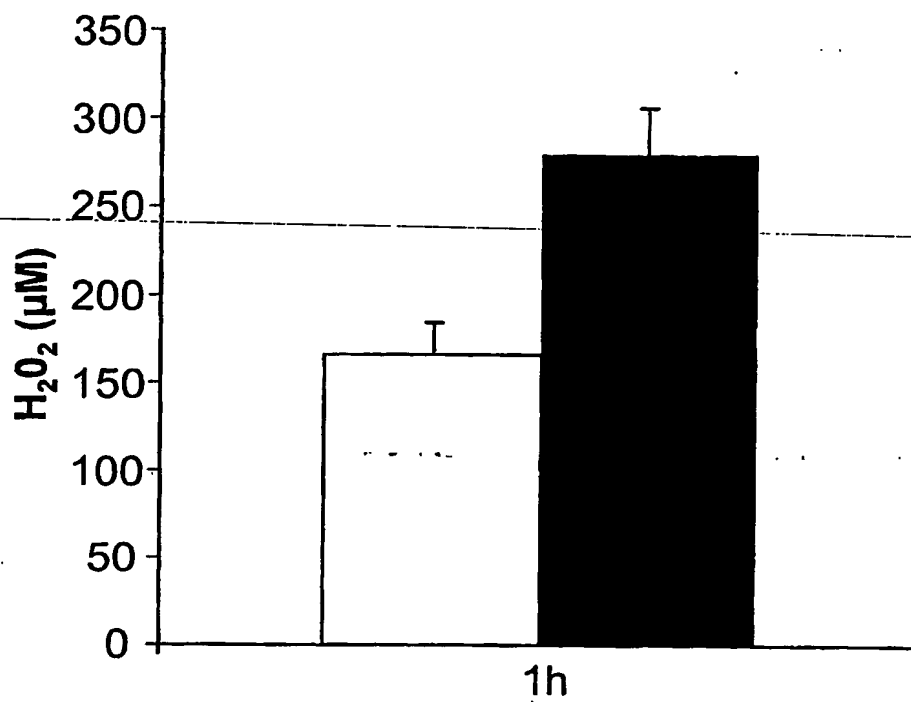
80



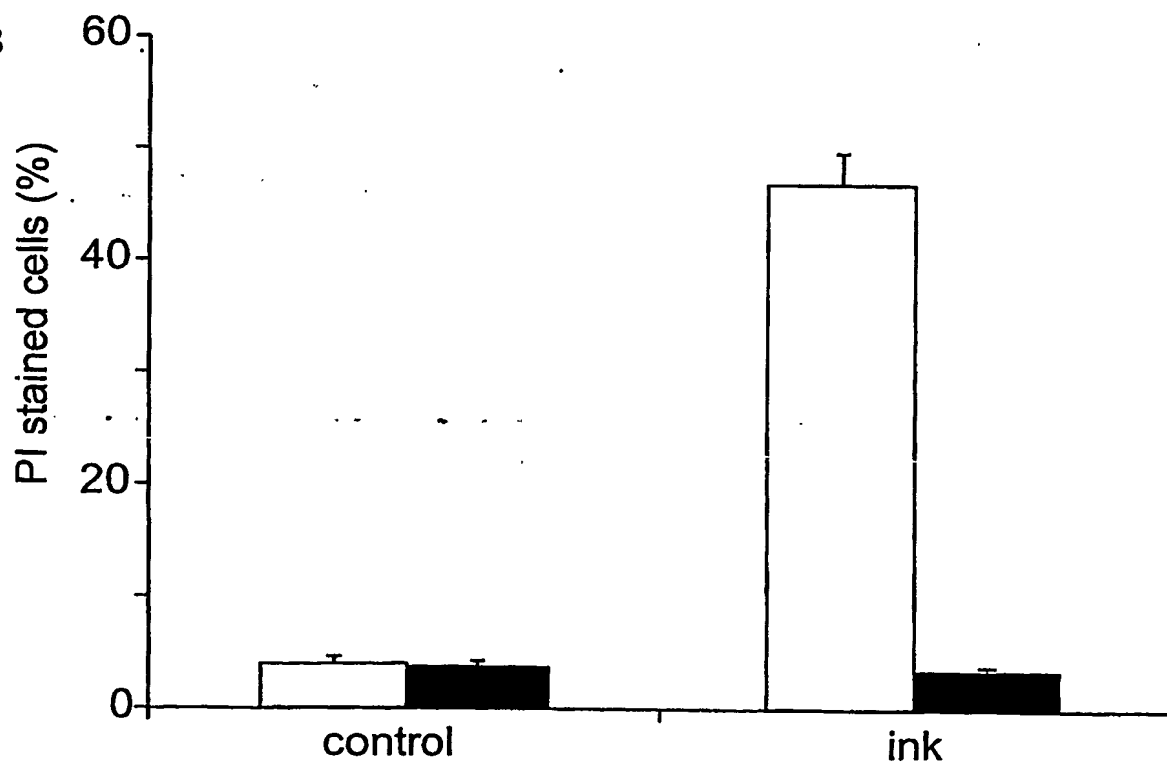
5 C

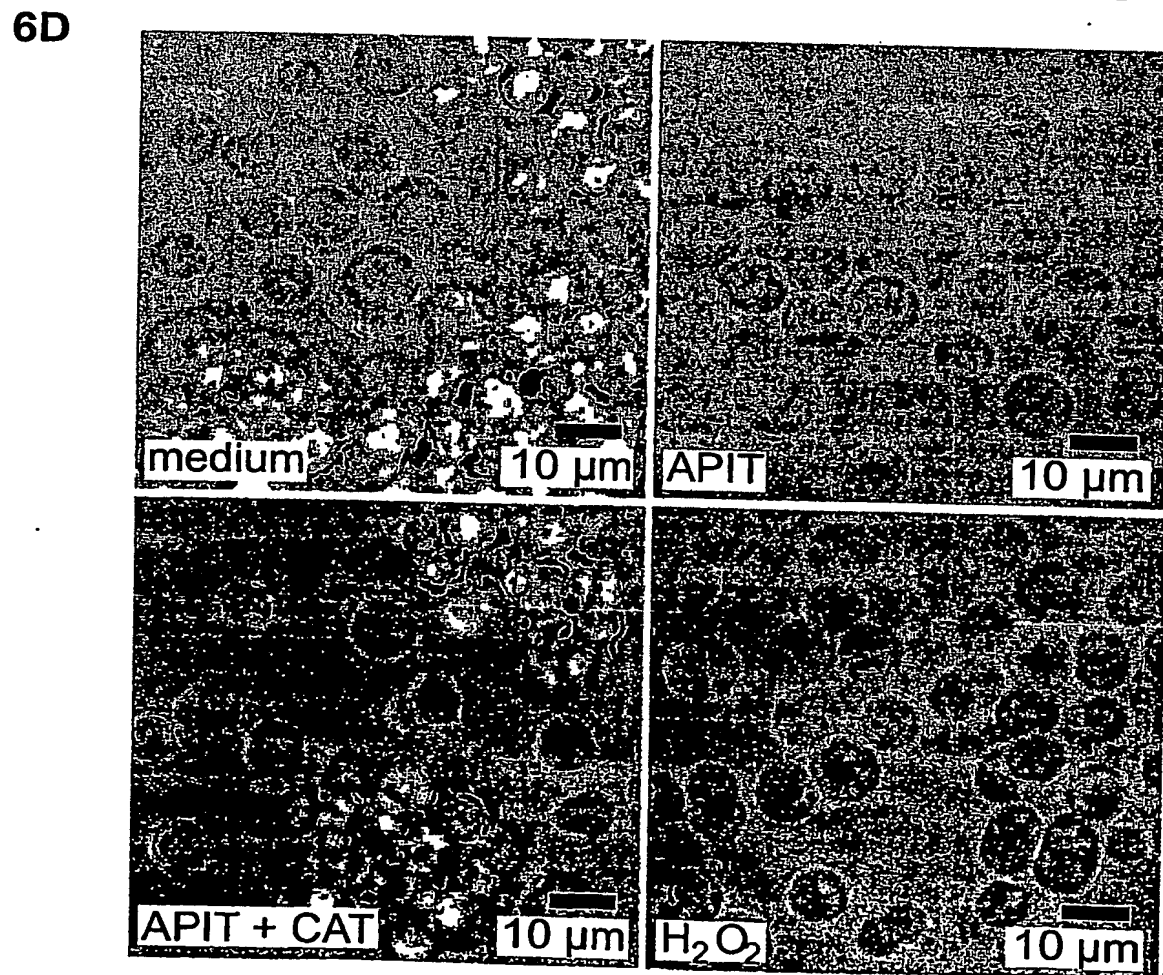
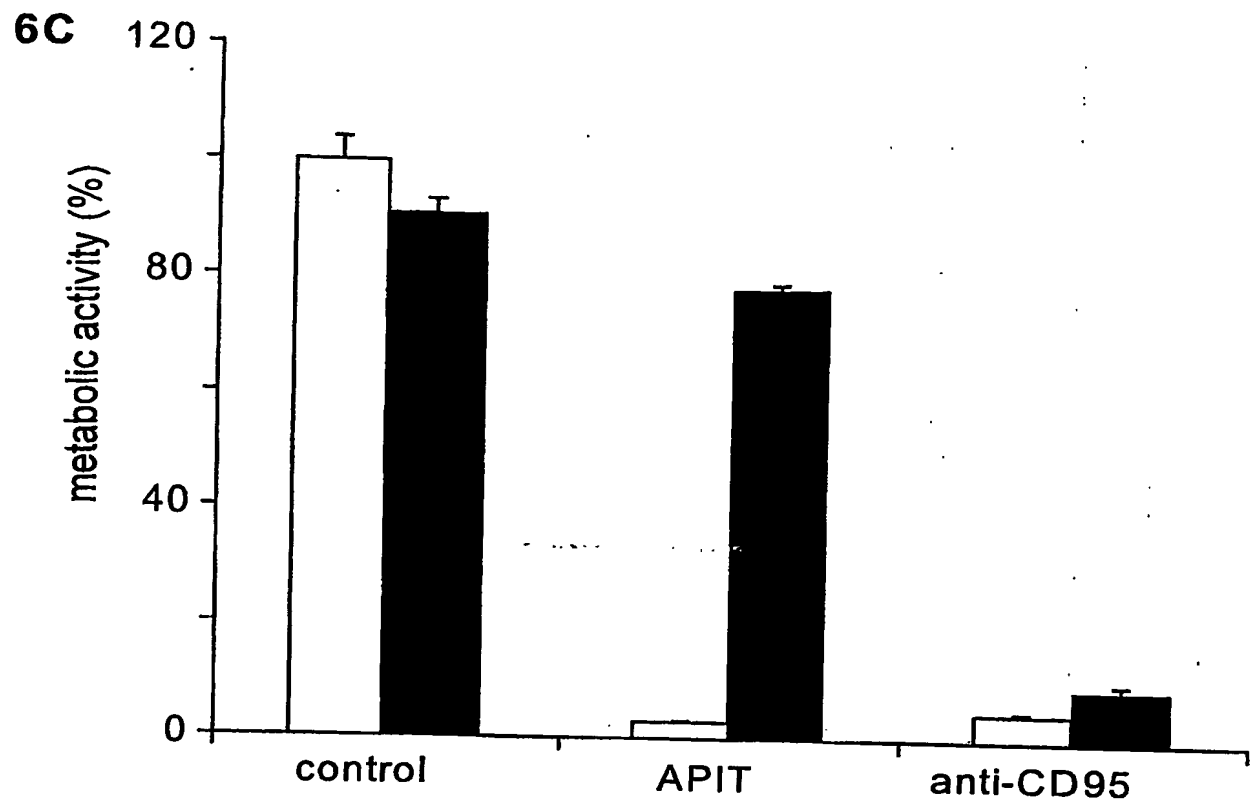


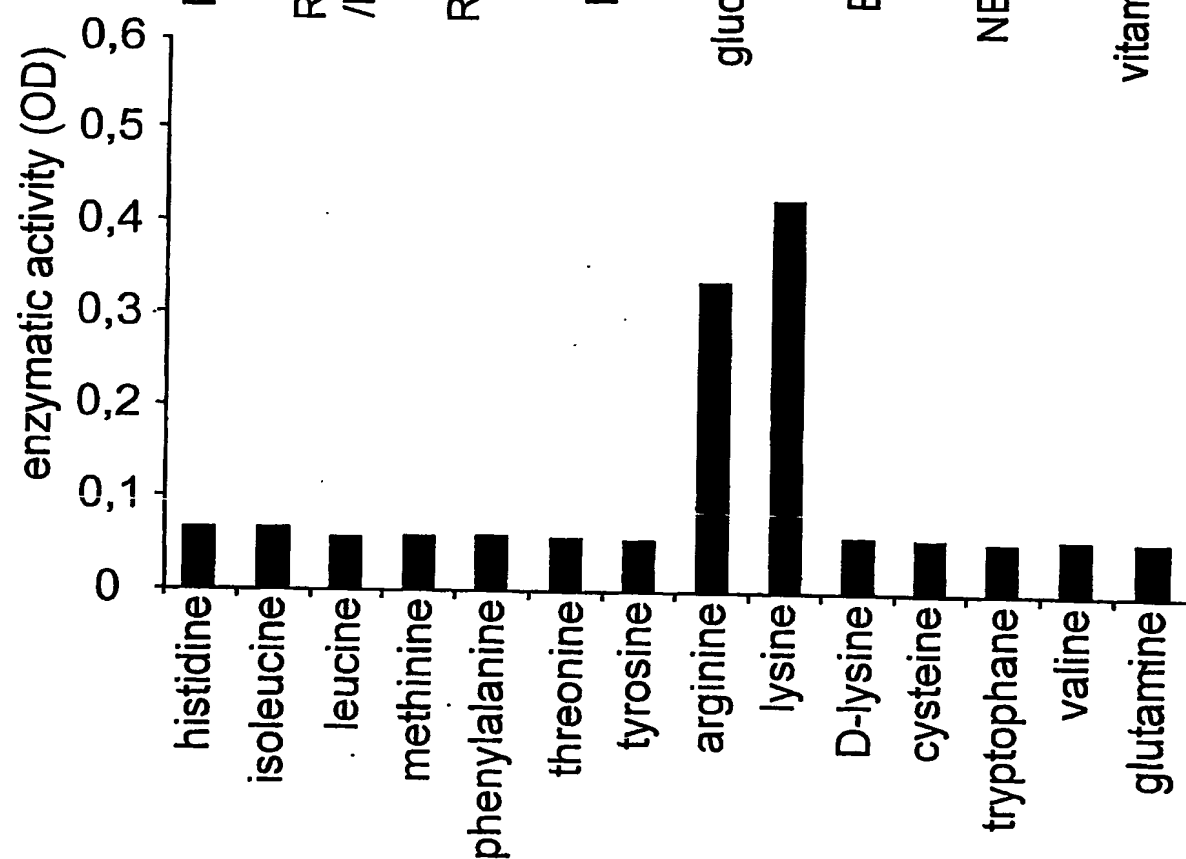
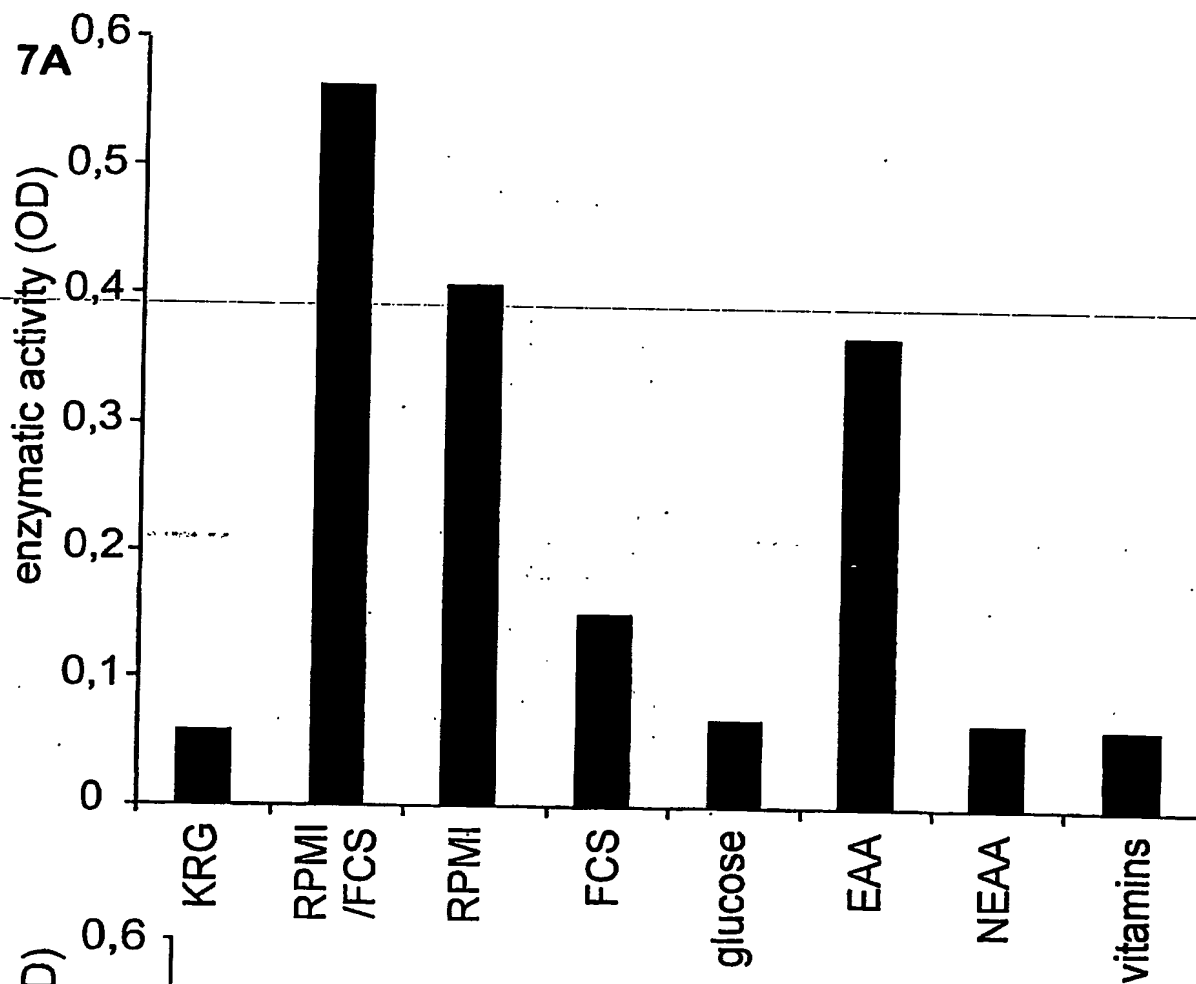
6A

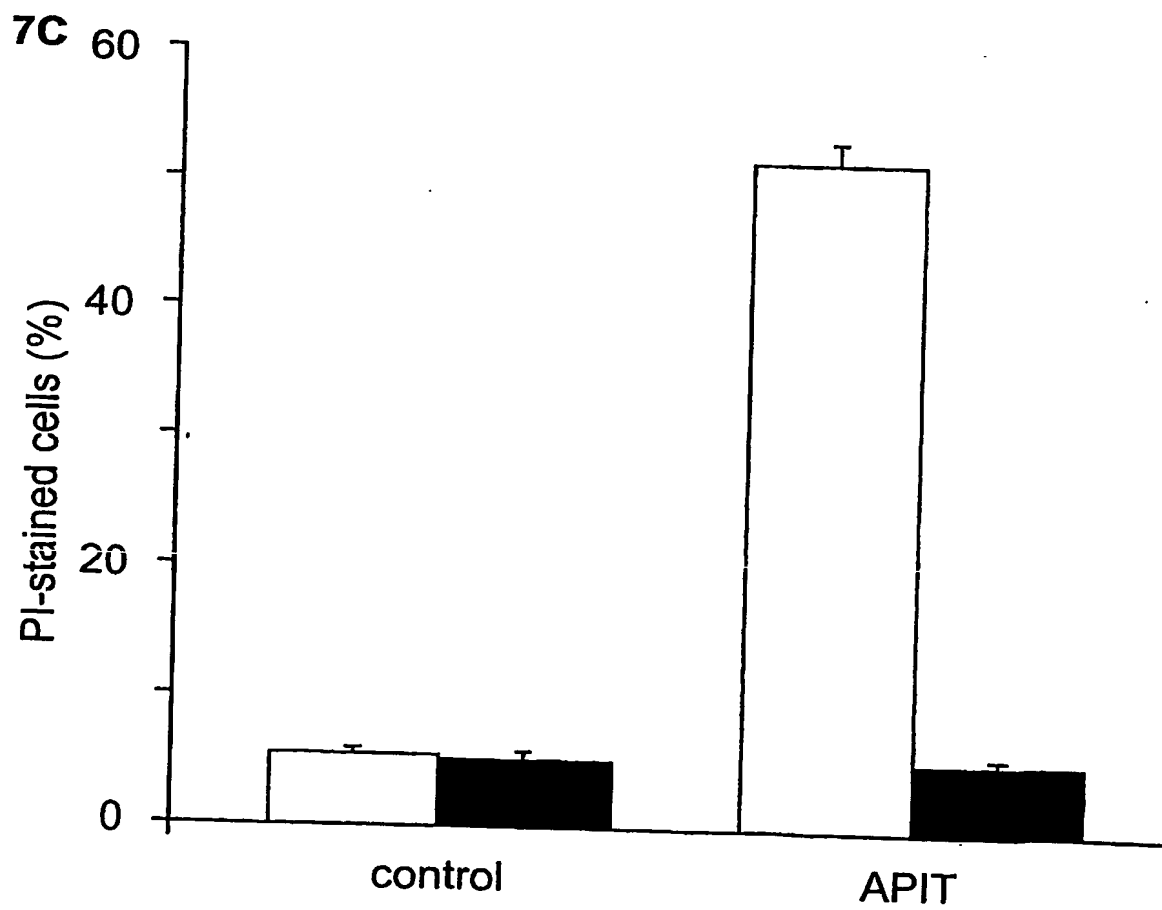
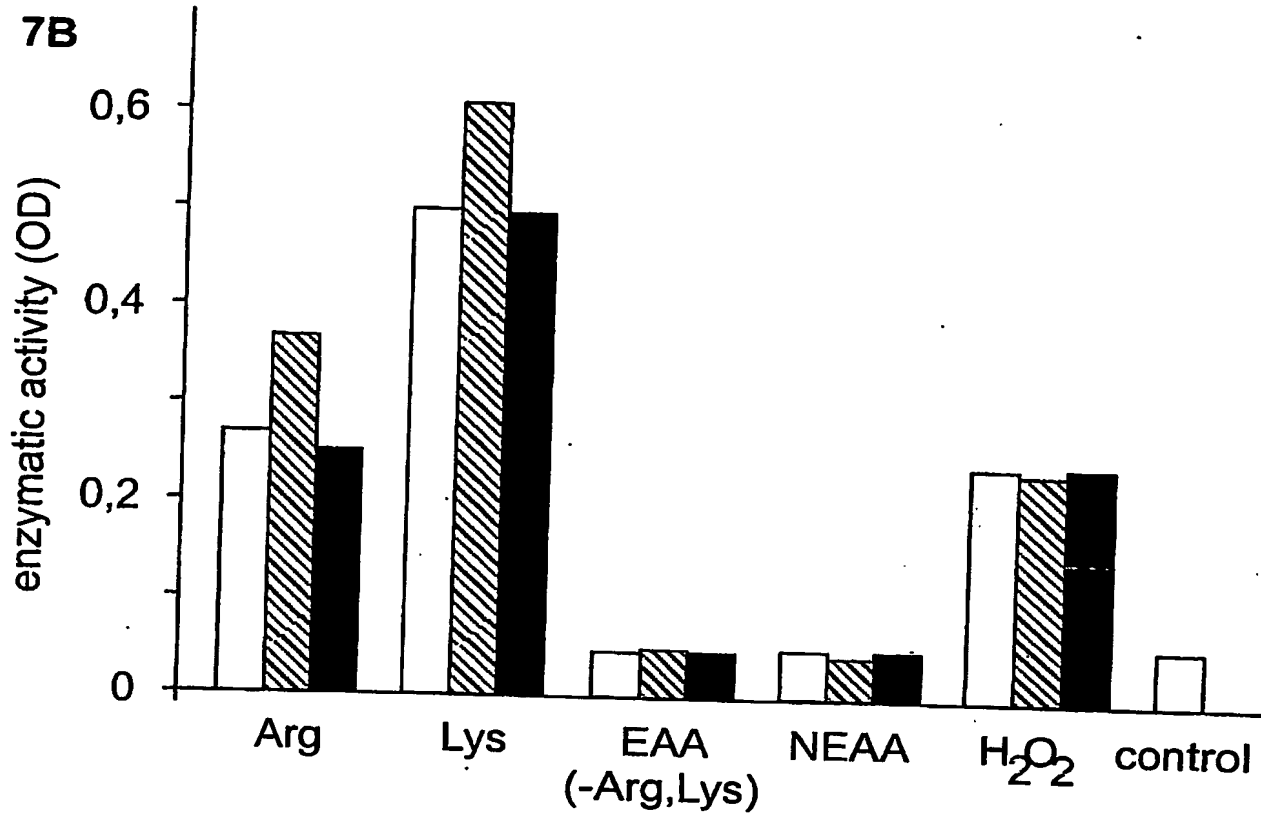


6B

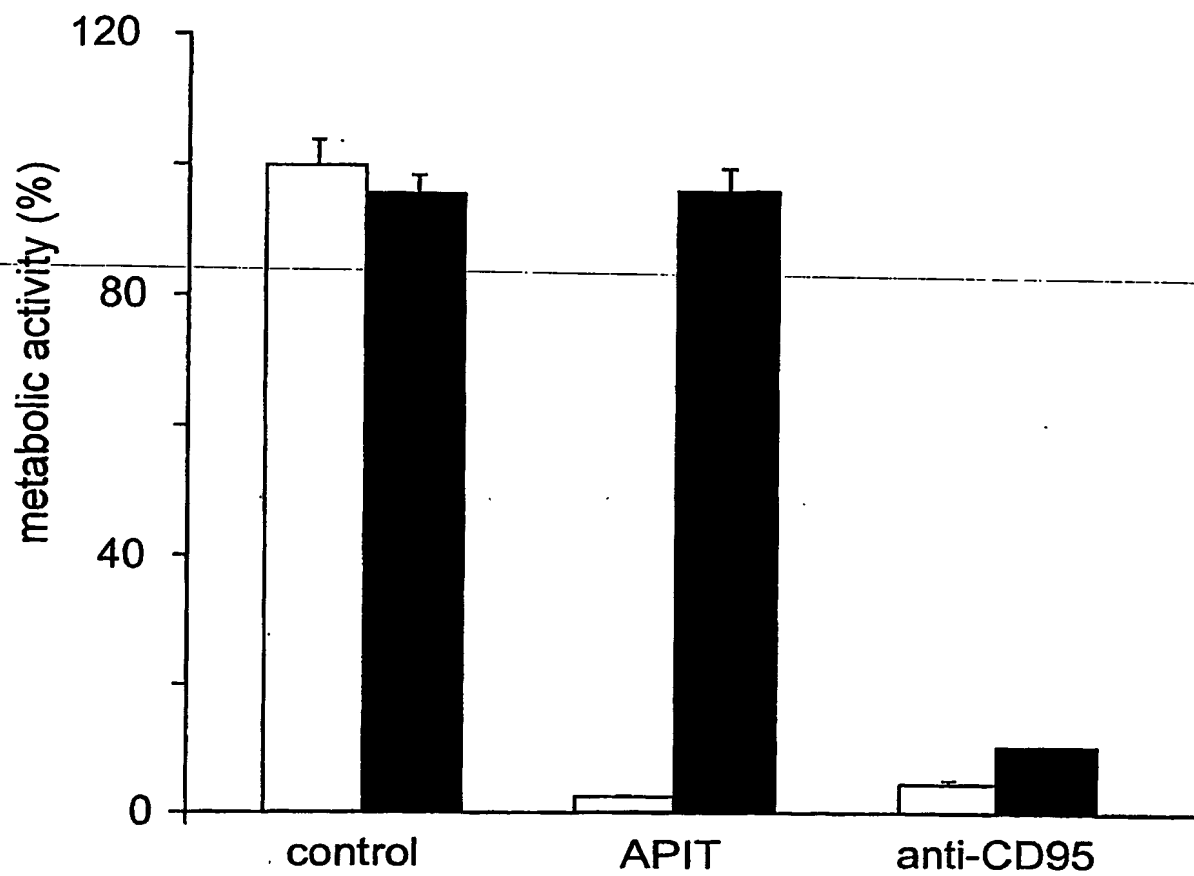




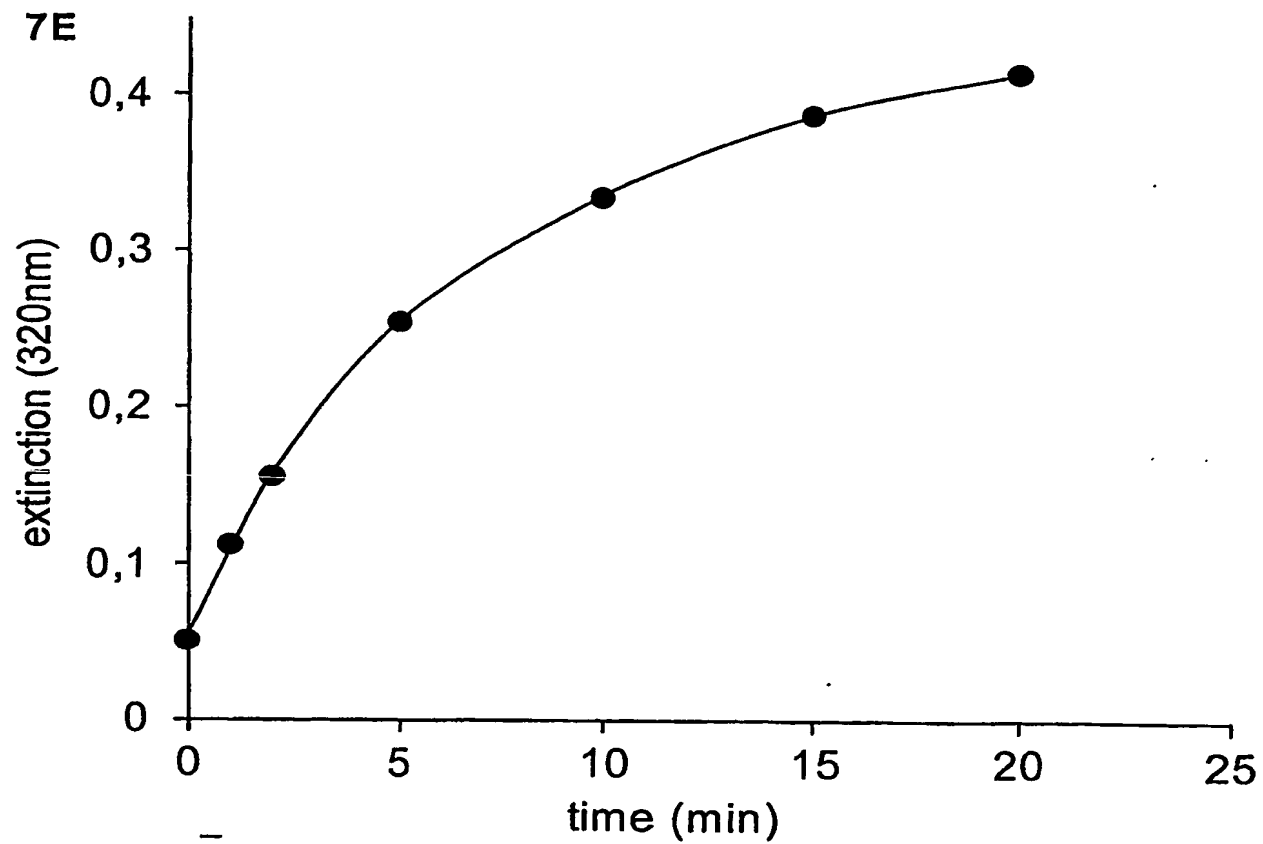




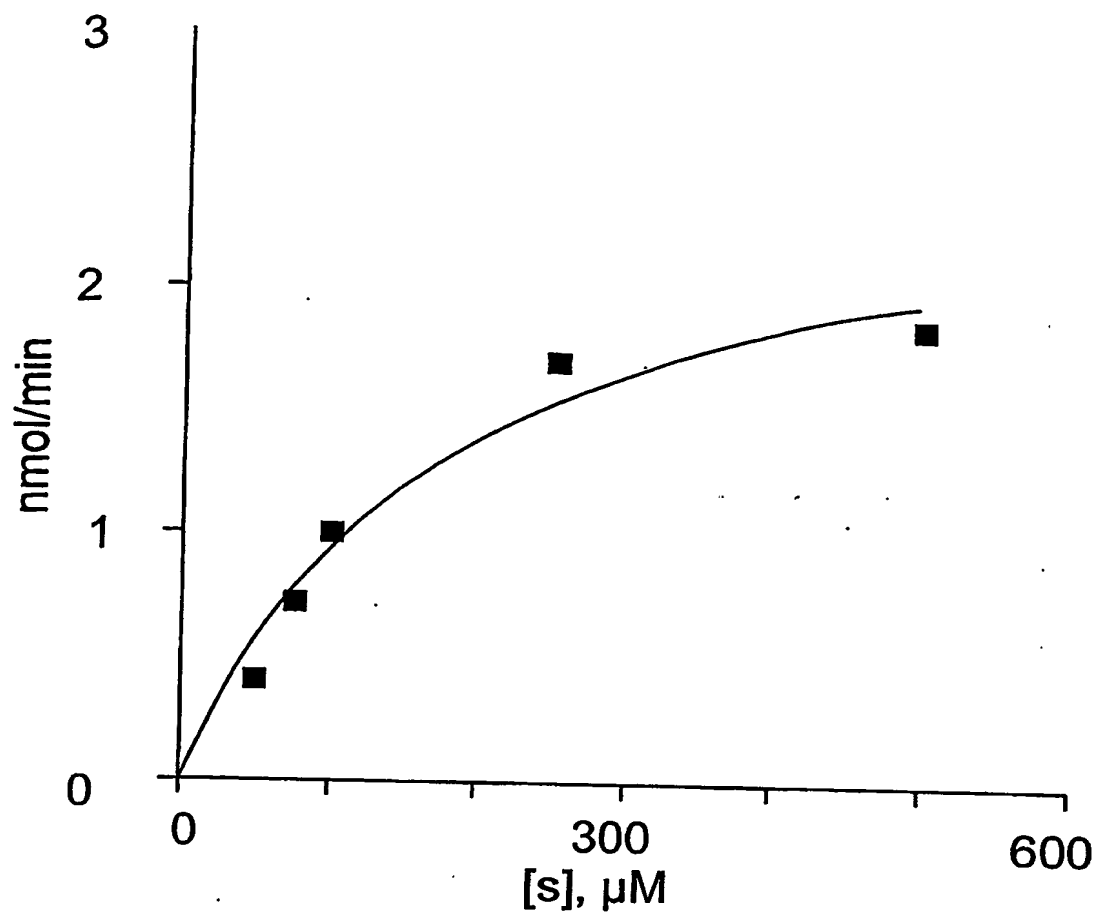
7D



7E



7F



7G

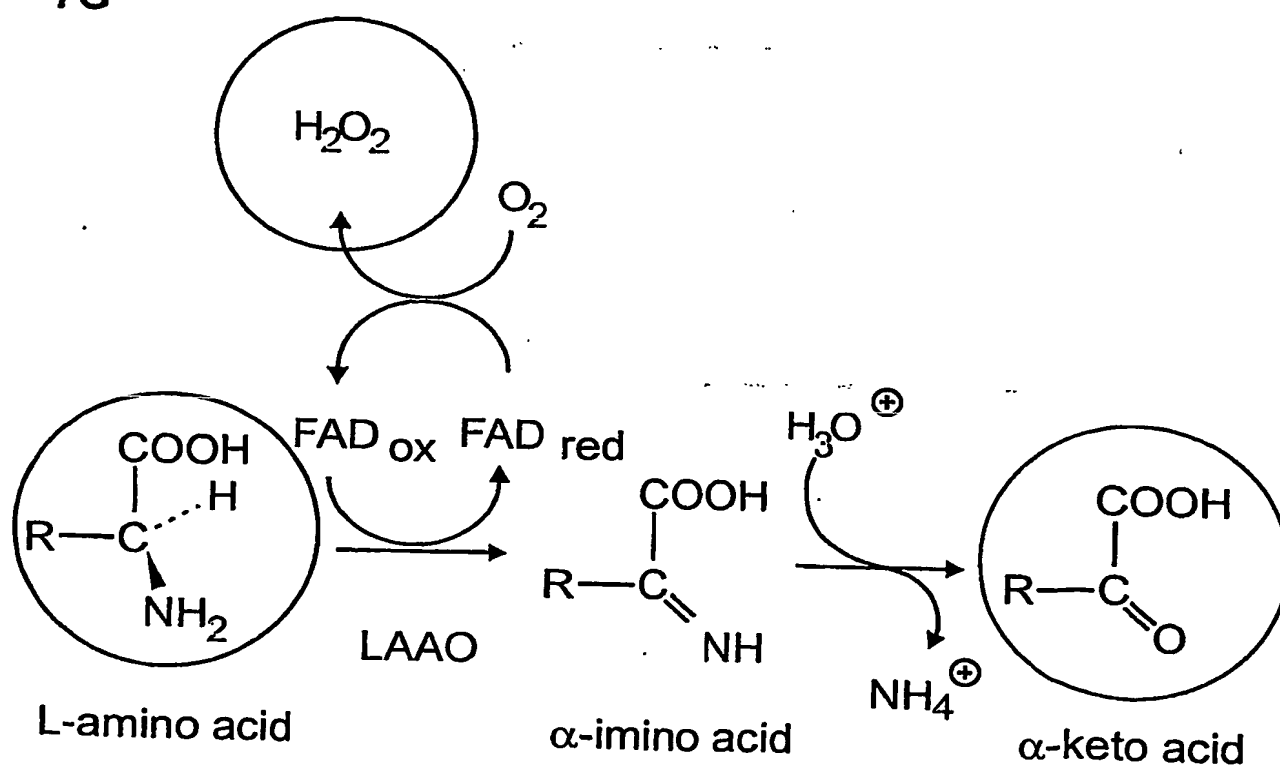


Fig. 8

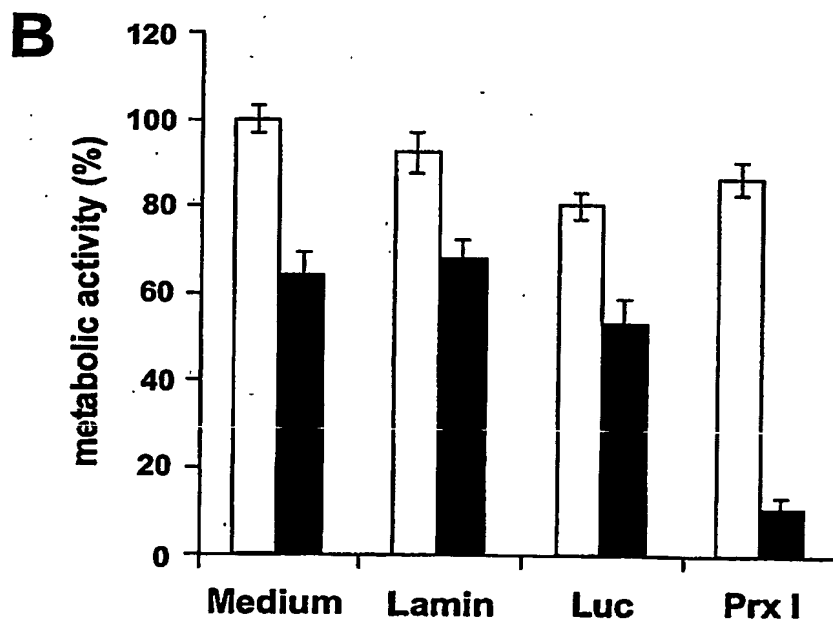
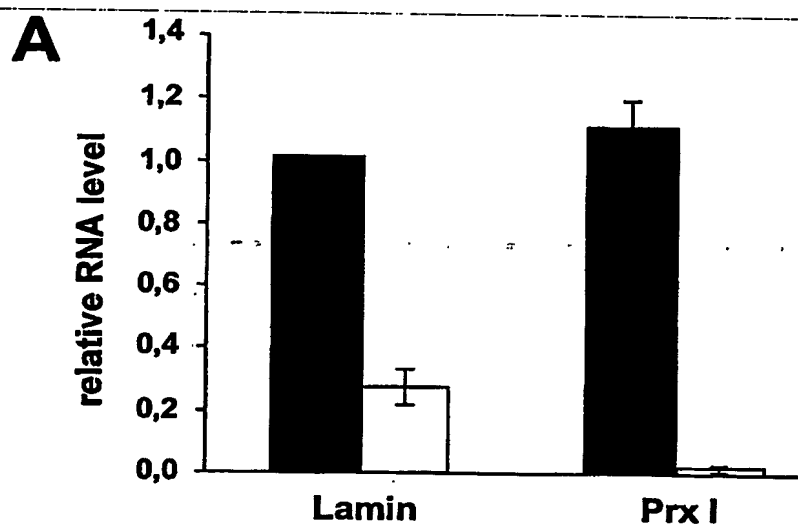


Fig. 9

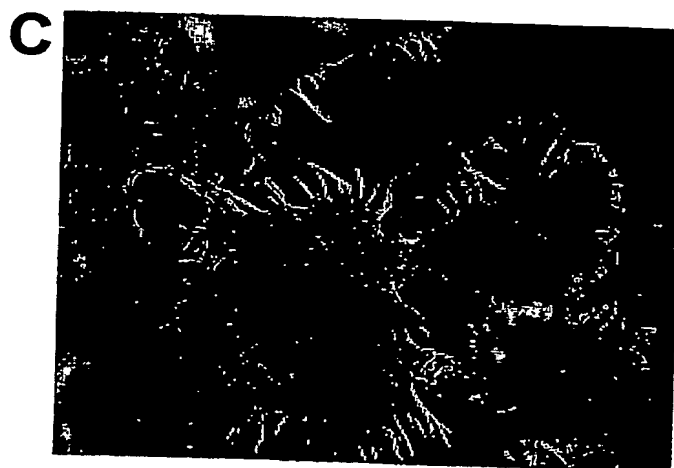
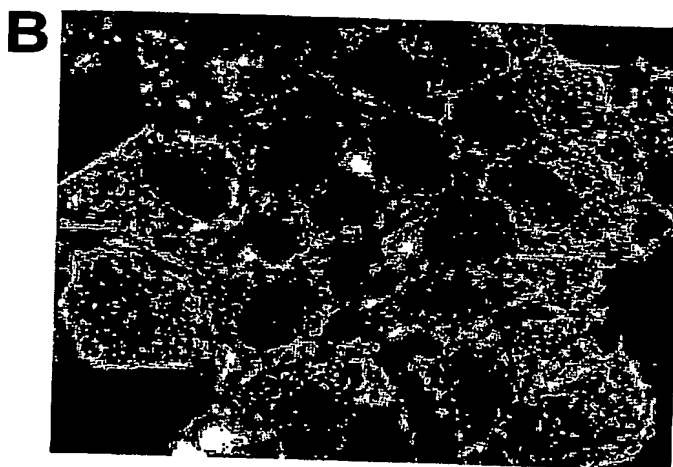
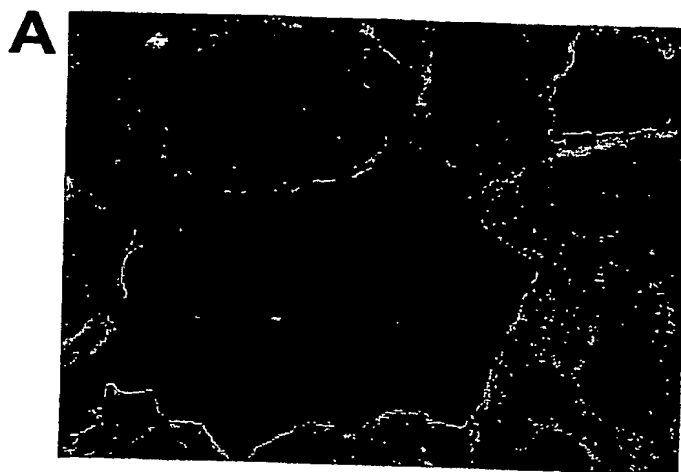
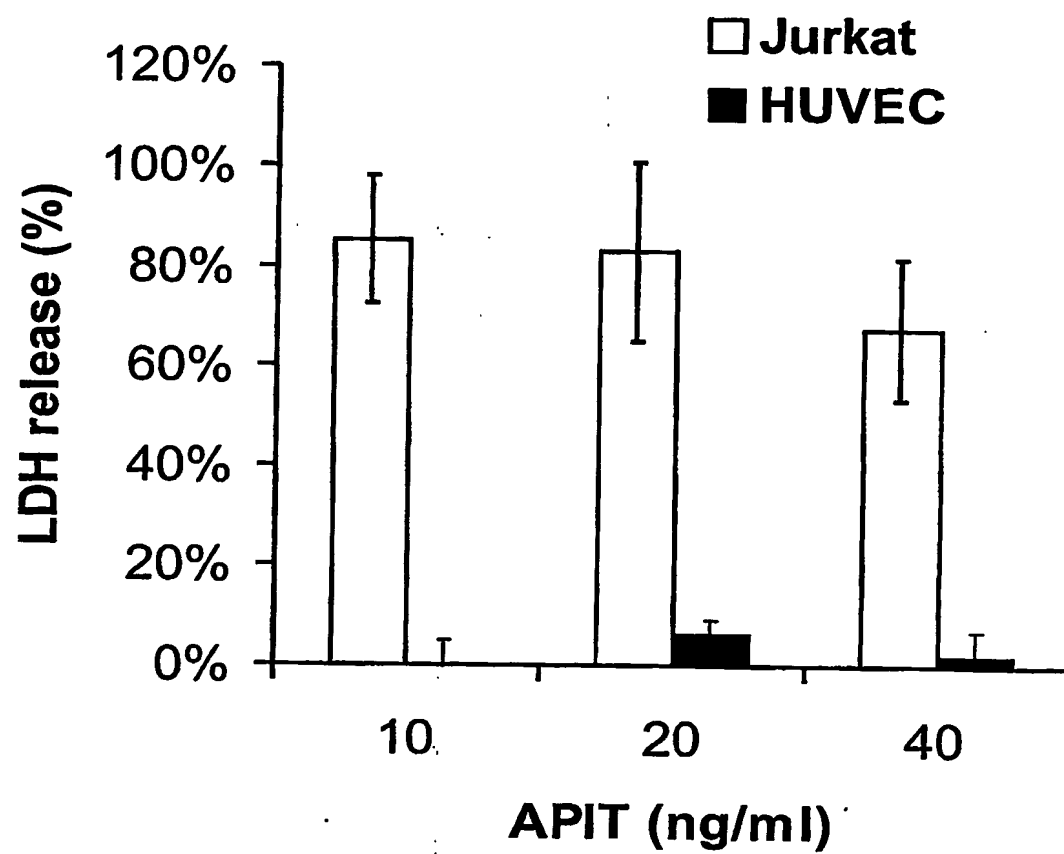


Fig. 10



SEQUENCE LISTING

<110> Max-Planck-Gesellschaft zur Förderung der Wissenc

<120> L-amino acid oxidase with cytotoxic activity from
Aplysia punctata

<130> 29644PEP-1

<140>

<141>

<160> 55

<170> PatentIn Ver. 2.1

<210> 1

<211> 1608

<212> DNA

<213> Aplysia punctata

<220>

<221> CDS

<222> (1)..(1608)

<400> 1

atg tcg tct gct gtg ctt ctc ctg gct tgt gcg ttg gtc atc tct gtc	48
Met Ser Ser Ala Val Leu Leu Leu Ala Cys Ala Leu Val Ile Ser Val	
1 5 10 15	
cac gcc gac ggt atc tgc aga aac aga cgt caa tgt aac aga gag gtg	96
His Ala Asp Gly Ile Cys Arg Asn Arg Arg Gln Cys Asn Arg Glu Val	
20 25 30	
tgc ggt tct acc tac gat gtg gcc gtc gtg ggg gcg ggg cct ggg gga	144
Cys Gly Ser Thr Tyr Asp Val Ala Val Val Gly Ala Gly Pro Gly Gly	
35 40 45	
gct aac tcc gcc tac atg ctg agg gac tcc ggc ctg gac atc gct gtg	192
Ala Asn Ser Ala Tyr Met Leu Arg Asp Ser Gly Leu Asp Ile Ala Val	
50 55 60	
ttc gag tac tcg gac cga gtg ggc ggc cgg ctg ttc acc tac cag ctg	240
Phe Glu Tyr Ser Asp Arg Val Gly Gly Arg Leu Phe Thr Tyr Gln Leu	
65 70 75 80	
ccc aac aca ccc gac gtt aac ctg gag att ggc ggc atg agg ttc atc	288
Pro Asn Thr Pro Asp Val Asn Leu Glu Ile Gly Gly Met Arg Phe Ile	
85 90 95	
gaa ggc gcc atg cac agg ctc tgg agg gtc att tca gaa ctc ggc cta	336
Glu Gly Ala Met His Arg Leu Trp Arg Val Ile Ser Glu Leu Gly Leu	
100 105 110	
acc ccc aag gtg ttc aag gaa ggt ttc ggc aag gag ggc aga caa aga	384
Thr Pro Lys Val Phe Lys Glu Gly Phe Gly Lys Glu Gly Arg Gln Arg	
115 120 125	
ttt tac ctg cgg gga cag agc ctg acc aag aaa cag gtc aag agt ggg	432
Phe Tyr Leu Arg Gly Gln Ser Leu Thr Lys Lys Gln Val Lys Ser Gly	
130 135 140	

gac gta ccc tat gac ctc agc ccg gag gag aaa gaa aac cag gga aat	480
Asp Val Pro Tyr Asp Leu Ser Pro Glu Glu Lys Glu Asn Gln Gly Asn	
145 150 155 160	
ctg gtc gaa tac tac ctg gag aaa ctg aca ggt cta caa ctc aac ggc	528
Leu Val Glu Tyr Tyr Leu Glu Lys Leu Thr Gly Leu Gln Leu Asn Gly	
165 170 175	
gag ccg ctc aaa cgt gag gtt gcg ctt aaa cta acc gtg ccg gac ggc	576
Glu Pro Leu Lys Arg Glu Val Ala Leu Lys Leu Thr Val Pro Asp Gly	
180 185 190	
aga ttc ctc tat gac ctc tcg ttt gac gaa gcc atg gat ctg gtt gcc	624
Arg Phe Leu Tyr Asp Leu Ser Phe Asp Glu Ala Met Asp Leu Val Ala	
195 200 205	
tcc cct gag ggc aaa gag ttc acc cga gac acg cac gtc ttc aca gga	672
Ser Pro Glu Gly Lys Glu Phe Thr Arg Asp Thr His Val Phe Thr Gly	
210 215 220	
gag gtc acc ctg gac gcg tcg gct gtc tcc ctc ttc gac gac cac ctg	720
Glu Val Thr Leu Asp Ala Ser Ala Val Ser Leu Phe Asp Asp His Leu	
225 230 235 240	
gga gag gac tac tat ggc agt gag atc tac acc cta aag gaa gga ctg	768
Gly Glu Asp Tyr Tyr Gly Ser Glu Ile Tyr Thr Leu Lys Glu Gly Leu	
245 250 255	
tct tcc gtc cca caa ggg ctc cta cag gct ttt ctg gac gcc gca gac	816
Ser Ser Val Pro Gln Gly Leu Leu Gln Ala Phe Leu Asp Ala Ala Asp	
260 265 270	
tcc aac gag ttc tat ccc aac agc cac ctg aag gcc ctg aga cgt aag	864
Ser Asn Glu Phe Tyr Pro Asn Ser His Leu Lys Ala Leu Arg Arg Lys	
275 280 285	
acc aac ggt cag tat gtt ctt tac ttt gag ccc acc acc tcc aag gat	912
Thr Asn Gly Gln Tyr Val Leu Tyr Phe Glu Pro Thr Thr Ser Lys Asp	
290 295 300	
gga caa acc aca atc aac tat ctg gaa ccc ctg cag gtt gtg tgt gca	960
Gly Gln Thr Thr Ile Asn Tyr Leu Glu Pro Leu Gln Val Val Cys Ala	
305 310 315 320	
caa aga gtc atc ctg gcc atg ccg gta tac gct ctg aac caa cta gac	1008
Gln Arg Val Ile Leu Ala Met Pro Val Tyr Ala Leu Asn Gln Leu Asp	
325 330 335	
tgg aat cag ctc aga aat gac cga gcc acc caa gcg tac gct gcc gtt	1056
Trp Asn Gln Leu Arg Asn Asp Arg Ala Thr Gln Ala Tyr Ala Val	
340 345 350	
cgc ccg att cct gca agt aag gtg ttc atg tcc ttt gat cag ccc tgg	1104
Arg Pro Ile Pro Ala Ser Lys Val Phe Met Ser Phe Asp Gln Pro Trp	
355 360 365	
tgg ttg gag aac gag agg aaa tcc tgg gtc acc aag tcg gac gcg ctt	1152
Trp Leu Glu Asn Glu Arg Lys Ser Trp Val Thr Lys Ser Asp Ala Leu	
370 375 380	

ttc agc caa atg tac gac tgg cag aag tct	gag gcg tcc gga gac tac	1200
Phe Ser Gln Met Tyr Asp Trp Gln Lys Ser	Glu Ala Ser Gly Asp Tyr	
385	390 395 400	
atc ctg atc gcc agc tac gcc gac ggc ctc	aaa gcc cag tac ctg cgg	1248
Ile Leu Ile Ala Ser Tyr Ala Asp Gly Leu	Lys Ala Gln Tyr Leu Arg	
405	410 415	
gag ctg aag aat cag gga gag gac atc cca	ggc tct gac cca ggc tac	1296
Glu Leu Lys Asn Gln Gly Glu Asp Ile Pro	Gly Ser Asp Pro Gly Tyr	
420	425 430	
aac cag gtt acc gaa ccc ctc aag gac acc	att ctt gac cac ctc act	1344
Asn Gln Val Thr Glu Pro Leu Lys Asp Thr	Ile Leu Asp His Leu Thr	
435	440 445	
gag gct tat ggc gtg gag cga gac tcg atc	ccg gaa ccc gtg acc gcc	1392
Glu Ala Tyr Gly Val Glu Arg Asp Ser Ile	Pro Glu Pro Val Thr Ala	
450	455 460	
gct tcc cag ttc tgg aca gac tac ccg ttt	ggc tgt gga tgg atc acc	1440
Ala Ser Gln Phe Trp Thr Asp Tyr Pro Phe	Gly Cys Gly Trp Ile Thr	
465	470 475 480	
tgg agg gcc ggc ttc cat ttc gat gac gtc	atc agc acc atg cgt cgc	1488
Trp Arg Ala Gly Phe His Phe Asp Asp Val	Ile Ser Thr Met Arg Arg	
485	490 495	
ccg tca ctg aaa gat gag gta tac gtg gtg	gga gcc gac tac tcc tgg	1536
Pro Ser Leu Lys Asp Glu Val Tyr Val Val	Gly Ala Asp Tyr Ser Trp	
500	505 510	
gga ctt atc tcc tcc tgg ata gag ggc gct	ctg gag acc tcg gaa aac	1584
Gly Leu Ile Ser Ser Trp Ile Glu Gly Ala	Leu Glu Thr Ser Glu Asn	
515	520 525	
gtc atc aac gac tac ttc ctc taa		1608
Val Ile Asn Asp Tyr Phe Leu		
530	535	

<210> 2
 <211> 535
 <212> PRT
 <213> Aplysia punctata

<400> 2
 Met Ser Ser Ala Val Leu Leu Leu Ala Cys Ala Leu Val Ile Ser Val
 1 5 10 15
 His Ala Asp Gly Ile Cys Arg Asn Arg Arg Gln Cys Asn Arg Glu Val
 20 25 30
 Cys Gly Ser Thr Tyr Asp Val Ala Val Val Gly Ala Gly Pro Gly Gly
 35 40 45
 Ala Asn Ser Ala Tyr Met Leu Arg Asp Ser Gly Leu Asp Ile Ala Val
 50 55 60
 Phe Glu Tyr Ser Asp Arg Val Gly Gly Arg Leu Phe Thr Tyr Gln Leu
 65 70 75 80

Ile Leu Ile Ala Ser Tyr Ala Asp Gly Leu Lys Ala Gln Tyr Leu Arg
 405 410 415
 Glu Leu Lys Asn Gln Gly Glu Asp Ile Pro Gly Ser Asp Pro Gly Tyr
 420 425 430
 Asn Gln Val Thr Glu Pro Leu Lys Asp Thr Ile Leu Asp His Leu Thr
 435 440 445
 Glu Ala Tyr Gly Val Glu Arg Asp Ser Ile Pro Glu Pro Val Thr Ala
 450 455 460
 Ala Ser Gln Phe Trp Thr Asp Tyr Pro Phe Gly Cys Gly Trp Ile Thr
 465 470 475 480
 Trp Arg Ala Gly Phe His Phe Asp Asp Val Ile Ser Thr Met Arg Arg
 485 490 495
 Pro Ser Leu Lys Asp Glu Val Tyr Val Val Gly Ala Asp Tyr Ser Trp
 500 505 510
 Gly Leu Ile Ser Ser Trp Ile Glu Gly Ala Leu Glu Thr Ser Glu Asn
 515 520 525
 Val Ile Asn Asp Tyr Phe Leu
 530 535

<210> 3
 <211> 1605
 <212> DNA
 <213> Aplysia punctata

<220>
 <221> CDS
 <222> (1)..(1605)

<220>
 <221> sig_peptide
 <222> (1)..(51)
 <223> Signal peptide not complete

<400> 3
 tcg tct gct gtg ctt ctc ctg gct tgt gcg ttg gtc atc tct gtc cac 48
 Ser Ser Ala Val Leu Leu Ala Cys Ala Leu Val Ile Ser Val His
 1 5 10 15
 gcc gac ggt gtc tgc aga aac aga cgt caa tgt aac aga gag gtg tgc 96
 Ala Asp Gly Val Cys Arg Asn Arg Arg Gln Cys Asn Arg Glu Val Cys
 20 25 30
 ggt tct acc tac gat gtg gcc gtc gtg ggg gcg ggg cct ggg gga gct 144
 Gly Ser Thr Tyr Asp Val Ala Val Val Gly Ala Gly Pro Gly Gly Ala
 35 40 45
 aac tcc gcc tac atg ctg agg gac tcc ggc ctg gac atc gct gtg ttc 192
 Asn Ser Ala Tyr Met Leu Arg Asp Ser Gly Leu Asp Ile Ala Val Phe
 50 55 60
 gag tac tca gac cga gtg ggc ggc cgg ctg ttc acc tac cag ctg ccc 240

Glu 65	Tyr	Ser	Asp	Arg	Val 70	Gly	Gly	Arg	Leu	Phe 75	Thr	Tyr	Gln	Leu	Pro 80	
aac Asn	aca Thr	ccc Pro	gac Asp	gtt Val	aat Asn	ctc Leu	gag Glu	att Ile	ggc Gly	ggc Gly	atg Met	agg Arg	ttc Phe	atc Ile	gag Glu	288
ggc Gly	gcc Ala	atg Met	cac His	agg Arg	ctc Leu	tgg Trp	agg Arg	gtc Val	att Ile	tca Ser	gaa Glu	ctc Leu	ggc Gly	cta Leu	acc Thr	336
ccc Pro	aag Lys	gtg Val	ttc Phe	aag Lys	gaa Glu	ggt Gly	ttc Phe	gga Gly	aag Lys	gag Glu	ggc Gly	aga Arg	cag Gln	aga Arg	ttt Phe	384
tac Tyr	ctg Leu	cgg Arg	gga Gly	cag Gln	agc Ser	ctg Leu	acc Thr	aag Lys	aaa Lys	cag Gln	gtc Val	aag Lys	agt Ser	ggg Gly	gac Asp	432
gta Val	ccc Pro	tat Tyr	gac Asp	ctc Leu	agc Ser	ccg Pro	gag Glu	gag Glu	aaa Lys	gaa Glu	aac Asn	cag Gln	gga Gly	aat Asn	ctg Leu	480
gtc Val	gaa Glu	tac Tyr	tac Tyr	ctg Leu	gag Glu	aaa Lys	ctg Leu	aca Thr	ggt Gly	cta Leu	caa Gln	ctc Leu	aat Asn	ggt Gly	gaa Glu	528
ccg Pro	ctc Leu	aaa Lys	cgt Arg	gag Glu	gtt Val	gcg Ala	ctt Leu	aaa Lys	cta Leu	acc Thr	gtg Val	ccg Pro	gac Asp	ggc Gly	aga Arg	576
ttc Phe	ctc Leu	tat Tyr	gac Asp	ctc Leu	tcg Ser	ttt Phe	gac Asp	gaa Glu	gcc Ala	atg Met	gat Asp	ctg Leu	gtt Val	gcc Ala	tcc Ser	624
cct Pro	gag Glu	ggc Gly	aaa Lys	gag Glu	ttc Phe	acc Thr	cga Arg	gac Asp	acg Thr	cac His	gtc Val	ttc Phe	acc Thr	gga Gly	gag Glu	672
gtc Val	acc Thr	ctg Leu	ggc Gly	gcg Ala	tcg Ser	gct Ala	gtc Val	tcc Ser	ctc Leu	ttc Phe	gac Asp	gac Asp	cac His	ctg Leu	gga Gly	720
gag Glu	gac Asp	tac Tyr	tac Tyr	ggc Gly	agt Ser	gag Glu	atc Ile	tac Tyr	acc Thr	ctc Leu	aag Lys	gaa Glu	gga Gly	ctg Leu	tct Ser	768
tcc Ser	gtc Val	cct Pro	caa Gln	ggg Gly	ctc Leu	cta Leu	cag Gln	gct Ala	ttt Phe	ctg Leu	gac Asp	gcc Ala	gca Ala	gac Asp	tcc Ser	816
aac Asn	gag Glu	ttc Phe	tat Tyr	ccc Pro	aac Asn	agc Ser	cac His	ctg Leu	aag Lys	gcc Ala	ctg Leu	aga Arg	cgt Arg	aag Lys	acc Thr	864
aac Asn	ggt Gly	cag Gln	tat Tyr	gtt Val	ctt Leu	tac Tyr	ttt Phe	gag Glu	ccc Pro	acc Thr	acc Thr	tcc Ser	aag Lys	gat Asp	gga Gly	912
caa Gln	acc Thr	aca Thr	atc Ile	aac Asn	tat Tyr	ctg Leu	gaa Glu	ccc Pro	ctg Leu	cag Gln	gtt Val	gtg Val	tgt Cys	gca Ala	cag Gln	960

305	310	315	320	
aga gtc att ctg gcc atg ccg gtc tac gct ctc aac cag ttg gat tgg				1008
Arg Val Ile Leu Ala Met Pro Val Tyr Ala Leu Asn Gln Leu Asp Trp				
325		330	335	
aat cag ctc aga aat gac cga gcc acc caa gcg tac gct gcc gtg cgc				1056
Asn Gln Leu Arg Asn Asp Arg Ala Thr Gln Ala Tyr Ala Ala Val Arg				
340		345	350	
ccg att cct gca agt aag gtg ttc atg acc ttt gat cag ccc tgg tgg				1104
Pro Ile Pro Ala Ser Lys Val Phe Met Thr Phe Asp Gln Pro Trp Trp				
355		360	365	
ttg gag aac gag agg aaa tcc tgg gtc acc aag tcg gac gcg ctt ttc				1152
Leu Glu Asn Glu Arg Lys Ser Trp Val Thr Lys Ser Asp Ala Leu Phe				
370		375	380	
agt caa atg tac gac tgg cag aag tct gag gcg tcc gga gac tac atc				1200
Ser Gln Met Tyr Asp Trp Gln Lys Ser Glu Ala Ser Gly Asp Tyr Ile				
385		390	395	400
ctg atc gcc agc tac gcc gac ggc ctc aaa gcc cag tac ctg cgg gag				1248
Leu Ile Ala Ser Tyr Ala Asp Gly Leu Lys Ala Gln Tyr Leu Arg Glu				
405		410	415	
ctg aag aat cag gga gag gac atc cca ggc tct gac cca ggc tac aac				1296
Leu Lys Asn Gln Gly Glu Asp Ile Pro Gly Ser Asp Pro Gly Tyr Asn				
420		425	430	
cag gtc acc gaa ccc ctc aag gac acc att ctt gac cac ctc act gag				1344
Gln Val Thr Glu Pro Leu Lys Asp Thr Ile Leu Asp His Leu Thr Glu				
435		440	445	
gcc tat ggc gtg gag cga gac tcg atc cgg gaa ccc gtg acc gcc gct				1392
Ala Tyr Gly Val Glu Arg Asp Ser Ile Arg Glu Pro Val Thr Ala Ala				
450		455	460	
tcc cag ttc tgg aca gac tac ccg ttt ggc tgt gga tgg atc acc tgg				1440
Ser Gln Phe Trp Thr Asp Tyr Pro Phe Gly Cys Gly Trp Ile Thr Trp				
465		470	475	480
agg gcc ggc ttc cat ttc gat gac gtc atc agc acc atg cgt cgc ccg				1488
Arg Ala Gly Phe His Phe Asp Asp Val Ile Ser Thr Met Arg Arg Pro				
485		490	495	
tca ctg aaa gat gag gtc tac gtg gtg gga gcc gat tac tcc tgg gga				1536
Ser Leu Lys Asp Glu Val Tyr Val Val Gly Ala Asp Tyr Ser Trp Gly				
500		505	510	
ctt atc tcc tcc tgg ata gag ggc gct ctg gag acc tca gaa aac gtc				1584
Leu Ile Ser Ser Trp Ile Glu Gly Ala Leu Glu Thr Ser Glu Asn Val				
515		520	525	
atc aac gac tac ttc ctc taa				1605
Ile Asn Asp Tyr Phe Leu				
530		535		

<210> 4
<211> 534

<212> PRT

<213> Aplysia punctata

<400> 4

Ser Ser Ala Val Leu Leu Leu Ala Cys Ala Leu Val Ile Ser Val His
1 5 10 15

Ala Asp Gly Val Cys Arg Asn Arg Arg Gln Cys Asn Arg Glu Val Cys
20 25 30

~~Gly Ser Thr Tyr Asp Val Ala Val Val Gly Ala Gly Pro Gly Gly Ala~~
~~35 40 45~~

Asn Ser Ala Tyr Met Leu Arg Asp Ser Gly Leu Asp Ile Ala Val Phe
50 55 60

Glu Tyr Ser Asp Arg Val Gly Gly Arg Leu Phe Thr Tyr Gln Leu Pro
65 70 75 80

Asn Thr Pro Asp Val Asn Leu Glu Ile Gly Gly Met Arg Phe Ile Glu
85 90 95

Gly Ala Met His Arg Leu Trp Arg Val Ile Ser Glu Leu Gly Leu Thr
100 105 110

Pro Lys Val Phe Lys Glu Gly Phe Gly Lys Glu Gly Arg Gln Arg Phe
115 120 125

Tyr Leu Arg Gly Gln Ser Leu Thr Lys Lys Gln Val Lys Ser Gly Asp
130 135 140

Val Pro Tyr Asp Leu Ser Pro Glu Glu Lys Glu Asn Gln Gly Asn Leu
145 150 155 160

Val Glu Tyr Tyr Leu Glu Lys Leu Thr Gly Leu Gln Leu Asn Gly Glu
165 170 175

Pro Leu Lys Arg Glu Val Ala Leu Lys Leu Thr Val Pro Asp Gly Arg
180 185 190

Phe Leu Tyr Asp Leu Ser Phe Asp Glu Ala Met Asp Leu Val Ala Ser
195 200 205

Pro Glu Gly Lys Glu Phe Thr Arg Asp Thr His Val Phe Thr Gly Glu
210 215 220

Val Thr Leu Gly Ala Ser Ala Val Ser Leu Phe Asp Asp His Leu Gly
225 230 235 240

Glu Asp Tyr Tyr Gly Ser Glu Ile Tyr Thr Leu Lys Glu Gly Leu Ser
245 250 255

Ser Val Pro Gln Gly Leu Leu Gln Ala Phe Leu Asp Ala Ala Asp Ser
260 265 270

Asn Glu Phe Tyr Pro Asn Ser His Leu Lys Ala Leu Arg Arg Lys Thr
275 280 285

Asn Gly Gln Tyr Val Leu Tyr Phe Glu Pro Thr Thr Ser Lys Asp Gly
290 295 300

Gln Thr Thr Ile Asn Tyr Leu Glu Pro Leu Gln Val Val Cys Ala Gln
 305 310 315 320
 Arg Val Ile Leu Ala Met Pro Val Tyr Ala Leu Asn Gln Leu Asp Trp
 325 330 335
 Asn Gln Leu Arg Asn Asp Arg Ala Thr Gln Ala Tyr Ala Ala Val Arg
 340 345 350
 Pro Ile Pro Ala Ser Lys Val Phe Met Thr Phe Asp Gln Pro Trp Trp
 355 360 365
 Leu Glu Asn Glu Arg Lys Ser Trp Val Thr Lys Ser Asp Ala Leu Phe
 370 375 380
 Ser Gln Met Tyr Asp Trp Gln Lys Ser Glu Ala Ser Gly Asp Tyr Ile
 385 390 395 400
 Leu Ile Ala Ser Tyr Ala Asp Gly Leu Lys Ala Gln Tyr Leu Arg Glu
 405 410 415
 Leu Lys Asn Gln Gly Glu Asp Ile Pro Gly Ser Asp Pro Gly Tyr Asn
 420 425 430
 Gln Val Thr Glu Pro Leu Lys Asp Thr Ile Leu Asp His Leu Thr Glu
 435 440 445
 Ala Tyr Gly Val Glu Arg Asp Ser Ile Arg Glu Pro Val Thr Ala Ala
 450 455 460
 Ser Gln Phe Trp Thr Asp Tyr Pro Phe Gly Cys Gly Trp Ile Thr Trp
 465 470 475 480
 Arg Ala Gly Phe His Phe Asp Asp Val Ile Ser Thr Met Arg Arg Pro
 485 490 495
 Ser Leu Lys Asp Glu Val Tyr Val Val Gly Ala Asp Tyr Ser Trp Gly
 500 505 510
 Leu Ile Ser Ser Trp Ile Glu Gly Ala Leu Glu Thr Ser Glu Asn Val
 515 520 525
 Ile Asn Asp Tyr Phe Leu
 530

<210> 5
 <211> 1554
 <212> DNA
 <213> *Aplysia punctata*

<220>
 <221> CDS
 <222> (1)..(1554)

<400> 5
 gac ggt atc tgc aga aac aga cgt caa tgt aac aga gag gtg tgc ggt 48
 Asp Gly Ile Cys Arg Asn Arg Arg Gln Cys Asn Arg Glu Val Cys Gly
 1 5 10 15

tct acc tac gat gtg gct gtc gtg ggg gcg ggg cct ggg gga gct aac	96
Ser Thr Tyr Asp Val Ala Val Val Gly Ala Gly Pro Gly Gly Ala Asn	
20 25 30	
tcc gcc tac atg ctg agg gac tcc ggc ctg gac atc gct gtg ttc gag	144
Ser Ala Tyr Met Leu Arg Asp Ser Gly Leu Asp Ile Ala Val Phe Glu	
35 40 45	
tac tca gac cga gtg ggc ggc cgg ctg ttc acc tac cag ctg ccc aac	192
Tyr Ser Asp Arg Val Gly Gly Arg Leu Phe Thr Tyr Gln Leu Pro Asn	
50 55 60	
aca ccc gac gtt aat ctc gag att ggc ggc atg agg ttc atc gag ggc	240
Thr Pro Asp Val Asn Leu Glu Ile Gly Gly Met Arg Phe Ile Glu Gly	
65 70 75 80	
gcc atg cac agg ctc tgg agg gtc att tca gaa ctc ggc cta acc ccc	288
Ala Met His Arg Leu Trp Arg Val Ile Ser Glu Leu Gly Leu Thr Pro	
85 90 95	
aag gtg ttc aag gaa ggt ttc gga aag gag ggc aga cag aga ttt tac	336
Lys Val Phe Lys Glu Gly Phe Gly Lys Glu Gly Arg Gln Arg Phe Tyr	
100 105 110	
ctg cgg gga cag agc ctg acc aag aaa cag gtc aag agt ggg gac gta	384
Leu Arg Gly Gln Ser Leu Thr Lys Lys Gln Val Lys Ser Gly Asp Val	
115 120 125	
ccc tat gac ctc agc ccg gag gag aaa gaa aac cag gga aat ctg gtc	432
Pro Tyr Asp Leu Ser Pro Glu Glu Lys Glu Asn Gln Gly Asn Leu Val	
130 135 140	
gaa tac tac ctg gag aaa ctg aca ggt cta aaa ctc aac ggc gga ccg	480
Glu Tyr Tyr Leu Glu Lys Leu Thr Gly Leu Lys Leu Asn Gly Gly Pro	
145 150 155 160	
ctc aaa cgt gag gtt gcg ctt aaa cta acc gtg ccg gac ggc aga ttc	528
Leu Lys Arg Glu Val Ala Leu Lys Leu Thr Val Pro Asp Gly Arg Phe	
165 170 175	
ctc tat gac ctc tcg ttt gac gaa gcc atg gac ctg gtt gcc tcc cct	576
Leu Tyr Asp Leu Ser Phe Asp Glu Ala Met Asp Leu Val Ala Ser Pro	
180 185 190	
gag ggc aaa gag ttc acc cga gac acg cac gtg ttc acc gga gaa gtc	624
Glu Gly Lys Glu Phe Thr Arg Asp Thr His Val Phe Thr Gly Glu Val	
195 200 205	
acc ctg gac gcg tcg gct gtc tcc ctc ttc gac gac cac ctg gga gag	672
Thr Leu Asp Ala Ser Ala Val Ser Leu Phe Asp Asp His Leu Gly Glu	
210 215 220	
gac tac tat ggc agt gag atc tac acc cta aag gaa gga ctg tct tcc	720
Asp Tyr Tyr Gly Ser Glu Ile Tyr Thr Leu Lys Glu Gly Leu Ser Ser	
225 230 235 240	
gtc cca caa ggg ctc cta cag act ttt ctg gac gcc gca gac tcc aac	768
Val Pro Gln Gly Leu Leu Gln Thr Phe Leu Asp Ala Ala Asp Ser Asn	
245 250 255	
gag ttc tat ccc aac agc cac ctg aag gcc ctg aga cgt aag acc aac	816

Glu Phe Tyr	Pro Asn Ser His Leu Lys Ala Leu Arg Arg Lys Thr Asn	
260	265	270
ggt cag tat gtt ctt tac ttt gag ccc acc acc tcc aag gat gga caa		864
Gly Gln Tyr Val Leu Tyr Phe Glu Pro Thr Thr Ser Lys Asp Gly Gln		
275	280	285
acc aca atc aac tat ctg gaa ccc ctg cag gtt gtg tgt gca cag aga		912
Thr Thr Ile Asn Tyr Leu Glu Pro Leu Gln Val Val Cys Ala Gln Arg		
290	295	300
gtc atc ctg gcc atg ccg gtc tac gct ctc aac caa ctg gac tgg aat		960
Val Ile Leu Ala Met Pro Val Tyr Ala Leu Asn Gln Leu Asp Trp Asn		
305	310	320
cag ctc aga aat gac cga gcc acc caa gcg tac gct gcc gtg cgc ccg		1008
Gln Leu Arg Asn Asp Arg Ala Thr Gln Ala Tyr Ala Ala Val Arg Pro		
325	330	335
att cct gca agt aaa gtg ttc atg acc ttt gat cag ccc tgg tgg ttg		1056
Ile Pro Ala Ser Lys Val Phe Met Thr Phe Asp Gln Pro Trp Trp Leu		
340	345	350
gag aac gag agg aaa tcc tgg gtc acc aag tcg gac gcg ctt ttc agc		1104
Glu Asn Glu Arg Lys Ser Trp Val Thr Lys Ser Asp Ala Leu Phe Ser		
355	360	365
caa atg tac gac tgg cag aag tct gag gcg tcc gga gac tac atc ctg		1152
Gln Met Tyr Asp Trp Gln Lys Ser Glu Ala Ser Gly Asp Tyr Ile Leu		
370	375	380
atc gcc agc tac gcc gac ggc ctc aaa gcc cag tac ctg cgg gag ctg		1200
Ile Ala Ser Tyr Ala Asp Gly Leu Lys Ala Gln Tyr Leu Arg Glu Leu		
385	390	400
aag aat cag gga gag gac atc cca ggc tct gac cca ggc tac aac cag		1248
Lys Asn Gln Gly Glu Asp Ile Pro Gly Ser Asp Pro Gly Tyr Asn Gln		
405	410	415
gtc acc gaa ccc ctc aag gac acc att ctt gac cac ctc act gag gct		1296
Val Thr Glu Pro Leu Lys Asp Thr Ile Leu Asp His Leu Thr Glu Ala		
420	425	430
tat ggc gtg gaa cga gac tcg atc ccg gaa ccc gtg acc gcc gct tcc		1344
Tyr Gly Val Glu Arg Asp Ser Ile Pro Glu Pro Val Thr Ala Ala Ser		
435	440	445
cag ttc tgg acc gac tac ccg ttc ggc tgt gga tgg atc acc tgg agg		1392
Gln Phe Trp Thr Asp Tyr Pro Phe Gly Cys Gly Trp Ile Thr Trp Arg		
450	455	460
gca ggc ttc cat ttt gat gac gtc atc agc acc atg cgt cgc ccg tca		1440
Ala Gly Phe His Phe Asp Asp Val Ile Ser Thr Met Arg Arg Pro Ser		
465	470	480
ctg aaa gat gag gtc tac gtg gtg gga gcc gat tac tcc tgg gga ctt		1488
Leu Lys Asp Glu Val Tyr Val Val Gly Ala Asp Tyr Ser Trp Gly Leu		
485	490	495
atc tcc tcc tgg ata gag ggc gct ctg gag acc tcg gaa aac gtc atc		1536
Ile Ser Ser Trp Ile Glu Gly Ala Leu Glu Thr Ser Glu Asn Val Ile		

500

505

510

aac gac tac ttc ctc taa
 Asn Asp Tyr Phe Leu
 515

1554

<210> 6

<211> 517

<212> PRT

<213> Aplysia punctata

<400> 6

Asp Gly Ile Cys Arg Asn Arg Arg Gln Cys Asn Arg Glu Val Cys Gly
 1 5 10 15

Ser Thr Tyr Asp Val Ala Val Val Gly Ala Gly Pro Gly Gly Ala Asn
 20 25 30

Ser Ala Tyr Met Leu Arg Asp Ser Gly Leu Asp Ile Ala Val Phe Glu
 35 40 45

Tyr Ser Asp Arg Val Gly Gly Arg Leu Phe Thr Tyr Gln Leu Pro Asn
 50 55 60

Thr Pro Asp Val Asn Leu Glu Ile Gly Gly Met Arg Phe Ile Glu Gly
 65 70 75 80

Ala Met His Arg Leu Trp Arg Val Ile Ser Glu Leu Gly Leu Thr Pro
 85 90 95

Lys Val Phe Lys Glu Gly Phe Gly Lys Glu Gly Arg Gln Arg Phe Tyr
 100 105 110

Leu Arg Gly Gln Ser Leu Thr Lys Lys Gln Val Lys Ser Gly Asp Val
 115 120 125

Pro Tyr Asp Leu Ser Pro Glu Glu Lys Glu Asn Gln Gly Asn Leu Val
 130 135 140

Glu Tyr Tyr Leu Glu Lys Leu Thr Gly Leu Lys Leu Asn Gly Gly Pro
 145 150 155 160

Leu Lys Arg Glu Val Ala Leu Lys Leu Thr Val Pro Asp Gly Arg Phe
 165 170 175

Leu Tyr Asp Leu Ser Phe Asp Glu Ala Met Asp Leu Val Ala Ser Pro
 180 185 190

Glu Gly Lys Glu Phe Thr Arg Asp Thr His Val Phe Thr Gly Glu Val
 195 200 205

Thr Leu Asp Ala Ser Ala Val Ser Leu Phe Asp Asp His Leu Gly Glu
 210 215 220

Asp Tyr Tyr Gly Ser Glu Ile Tyr Thr Leu Lys Glu Gly Leu Ser Ser
 225 230 235 240

Val Pro Gln Gly Leu Leu Gln Thr Phe Leu Asp Ala Ala Asp Ser Asn
 245 250 255

Glu Phe Tyr Pro Asn Ser His Leu Lys Ala Leu Arg Arg Lys Thr Asn
 260 265 270
 Gly Gln Tyr Val Leu Tyr Phe Glu Pro Thr Thr Ser Lys Asp Gly Gln
 275 280 285
 Thr Thr Ile Asn Tyr Leu Glu Pro Leu Gln Val Val Cys Ala Gln Arg
 290 295 300
 Val Ile Leu Ala Met Pro Val Tyr Ala Leu Asn Gln Leu Asp Trp Asn
 305 310 315 320
 Gln Leu Arg Asn Asp Arg Ala Thr Gln Ala Tyr Ala Ala Val Arg Pro
 325 330 335
 Ile Pro Ala Ser Lys Val Phe Met Thr Phe Asp Gln Pro Trp Trp Leu
 340 345 350
 Glu Asn Glu Arg Lys Ser Trp Val Thr Lys Ser Asp Ala Leu Phe Ser
 355 360 365
 Gln Met Tyr Asp Trp Gln Lys Ser Glu Ala Ser Gly Asp Tyr Ile Leu
 370 375 380
 Ile Ala Ser Tyr Ala Asp Gly Leu Lys Ala Gln Tyr Leu Arg Glu Leu
 385 390 395 400
 Lys Asn Gln Gly Glu Asp Ile Pro Gly Ser Asp Pro Gly Tyr Asn Gln
 405 410 415
 Val Thr Glu Pro Leu Lys Asp Thr Ile Leu Asp His Leu Thr Glu Ala
 420 425 430
 Tyr Gly Val Glu Arg Asp Ser Ile Pro Glu Pro Val Thr Ala Ala Ser
 435 440 445
 Gln Phe Trp Thr Asp Tyr Pro Phe Gly Cys Gly Trp Ile Thr Trp Arg
 450 455 460
 Ala Gly Phe His Phe Asp Asp Val Ile Ser Thr Met Arg Arg Pro Ser
 465 470 475 480
 Leu Lys Asp Glu Val Tyr Val Val Gly Ala Asp Tyr Ser Trp Gly Leu
 485 490 495
 Ile Ser Ser Trp Ile Glu Gly Ala Leu Glu Thr Ser Glu Asn Val Ile
 500 505 510
 Asn Asp Tyr Phe Leu
 515

<210> 7
 <211> 600
 <212> DNA
 <213> Human

 <220>
 <221> CDS
 <222> (1)..(600)

<400> 7

atg tct tca gga aat gct aaa att ggg cac cct gcc ccc aac ttc aaa 48
Met Ser Ser Gly Asn Ala Lys Ile Gly His Pro Ala Pro Asn Phe Lys
1 5 10 15

gcc aca gct gtt atg cca gat ggt cag ttt aaa gat atc agc ctg tct 96
Ala Thr Ala Val Met Pro Asp Gly Gln Phe Lys Asp Ile Ser Leu Ser
20 25 30

~~gac tac aaa gga aaa tat gtt gtg ttc ttc ttt tac cct ctt gac ttc 144~~
~~Asp Tyr Lys Gly Lys Tyr Val Val Phe Phe Phe Tyr Pro Leu Asp Phe~~
~~35 40 45~~

acc ttt gtg tgc ccc acg gag atc att gct ttc agt gat agg gca gaa 192
Thr Phe Val Cys Pro Thr Glu Ile Ile Ala Phe Ser Asp Arg Ala Glu
50 55 60

gaa ttt aag aaa ctc aac tgc caa gtg att ggt gct tct gtg gat tct 240
Glu Phe Lys Lys Leu Asn Cys Gln Val Ile Gly Ala Ser Val Asp Ser
65 70 75 80

cac ttc tgt cat cta gca tgg gtc aat aca cct aag aaa caa gga gga 288
His Phe Cys His Leu Ala Trp Val Asn Thr Pro Lys Lys Gln Gly Gly
85 90 95

ctg gga ccc atg aac att cct ttg gta tca gac ccg aag cgc acc att 336
Leu Gly Pro Met Asn Ile Pro Leu Val Ser Asp Pro Lys Arg Thr Ile
100 105 110

gct cag gat tat ggg gtc tta aag gct gat gaa ggc atc tcg ttc agg 384
Ala Gln Asp Tyr Gly Val Leu Lys Ala Asp Glu Gly Ile Ser Phe Arg
115 120 125

ggc ctt ttt atc att gat gat aag ggt att ctt cgg cag atc act gta 432
Gly Leu Phe Ile Ile Asp Asp Lys Gly Ile Leu Arg Gln Ile Thr Val
130 135 140

aat gac ctc cct gtt ggc cgc tct gtg gat gag act ttg aga cta gtt 480
Asn Asp Leu Pro Val Gly Arg Ser Val Asp Glu Thr Leu Arg Leu Val
145 150 155 160

cag gcc ttc cag ttc act gac aaa cat ggg gaa gtg tgc cca gct ggc 528
Gln Ala Phe Gln Phe Thr Asp Lys His Gly Glu Val Cys Pro Ala Gly
165 170 175

tgg aaa cct ggc agt gat acc atc aag cct gat gtc caa aag agc aaa 576
Trp Lys Pro Gly Ser Asp Thr Ile Lys Pro Asp Val Gln Lys Ser Lys
180 185 190

gaa tat ttc tcc aag cag aag tga 600
Glu Tyr Phe Ser Lys Gln Lys
195 200

<210> 8

<211> 199

<212> PRT

<213> Human

<400> 8

Met Ser Ser Gly Asn Ala Lys Ile Gly His Pro Ala Pro Asn Phe Lys
 1 5 10 15
 Ala Thr Ala Val Met Pro Asp Gly Gln Phe Lys Asp Ile Ser Leu Ser
 20 25 30
 Asp Tyr Lys Gly Lys Tyr Val Val Phe Phe Phe Tyr Pro Leu Asp Phe
 35 40 45
 Thr Phe Val Cys Pro Thr Glu Ile Ile Ala Phe Ser Asp Arg Ala Glu
 50 55 60
 Glu Phe Lys Lys Leu Asn Cys Gln Val Ile Gly Ala Ser Val Asp Ser
 65 70 75 80
 His Phe Cys His Leu Ala Trp Val Asn Thr Pro Lys Lys Gln Gly Gly
 85 90 95
 Leu Gly Pro Met Asn Ile Pro Leu Val Ser Asp Pro Lys Arg Thr Ile
 100 105 110
 Ala Gln Asp Tyr Gly Val Leu Lys Ala Asp Glu Gly Ile Ser Phe Arg
 115 120 125
 Gly Leu Phe Ile Ile Asp Asp Lys Gly Ile Leu Arg Gln Ile Thr Val
 130 135 140
 Asn Asp Leu Pro Val Gly Arg Ser Val Asp Glu Thr Leu Arg Leu Val
 145 150 155 160
 Gln Ala Phe Gln Phe Thr Asp Lys His Gly Glu Val Cys Pro Ala Gly
 165 170 175
 Trp Lys Pro Gly Ser Asp Thr Ile Lys Pro Asp Val Gln Lys Ser Lys
 180 185 190
 Glu Tyr Phe Ser Lys Gln Lys
 195

<210> 9
 <211> 19
 <212> RNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: synthetic
 double-stranded RNA molecule

<400> 9
 ggcaugaa ggcaucug

19

<210> 10
 <211> 19
 <212> RNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: synthetic

double-stranded RNA molecule

<400> 10
augcuaaaau ugggcacccc 19

<210> 11
<211> 19
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic
double-stranded RNA molecule

<400> 11
ugcuaaaau gggcacccu 19

<210> 12
<211> 19
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic
double-stranded RNA molecule

<400> 12
cuucaaagcc acagcuguu 19

<210> 13
<211> 19
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic
double-stranded RNA molecule

<400> 13
agccacagcu guuaugcca 19

<210> 14
<211> 19
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic
double-stranded RNA molecule

<400> 14
gccacagcug uuaugccag 19

<210> 15
<211> 19
<212> RNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic
double-stranded RNA molecule

<400> 15

agauaucagc cugucugac

19

<210> 16

<211> 19

<212> RNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic
double-stranded RNA molecule

<400> 16

gauaucagcc ugucugacu

19

<210> 17

<211> 19

<212> RNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic
double-stranded RNA molecule

<400> 17

gaaacucaac ugccaagug

19

<210> 18

<211> 19

<212> RNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic
double-stranded RNA molecule

<400> 18

acucaacugc caagugauu

19

<210> 19

<211> 19

<212> RNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic
double-stranded RNA molecule

<400> 19

cucaacugcc aagugauug

19

<210> 20
 <211> 19
 <212> RNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: synthetic
 double-stranded RNA molecule

<400> 20
 cugccaagug auuggugcu 19

<210> 21
 <211> 19
 <212> RNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: synthetic
 double-stranded RNA molecule

<400> 21
 gugauuggug cuucugugg 19

<210> 22
 <211> 19
 <212> RNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: synthetic
 double-stranded RNA molecule

<400> 22
 gaaacaagga ggacuggga 19

<210> 23
 <211> 19
 <212> RNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: synthetic
 double-stranded RNA molecule

<400> 23
 cauuccuuug guaucagac 19

<210> 24
 <211> 19
 <212> RNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: synthetic
 double-stranded RNA molecule

<400> 24
aggcugauga aggcaucuc

19

<210> 25
<211> 19
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic
double-stranded RNA molecule

<400> 25
gcgcaccauu gcucaggau

19

<210> 26
<211> 19
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic
double-stranded RNA molecule

<400> 26
ggguauucuu cggcagauc

19

<210> 27
<211> 19
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic
double-stranded RNA molecule

<400> 27
accuggcagu gauaccauc

19

<210> 28
<211> 19
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic
double-stranded RNA molecule

<400> 28
ccuggcagug auaccauca

19

<210> 29
<211> 19
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic
double-stranded RNA molecule

<400> 29
gccugauguc caaaagagc

19

<210> 30
<211> 19
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic
double-stranded RNA molecule

<400> 30
cuggacuucc agaagaaca

19

<210> 31
<211> 19
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic
double-stranded RNA molecule

<400> 31
cuuacgcuga guacuucga

19

<210> 32
<211> 7
<212> PRT
<213> Aplysia

<400> 32
Asp Gly Glu Asp Ala Ala Val
1 5

<210> 33
<211> 9
<212> PRT
<213> Aplysia

<220>
<221> MOD_RES
<222> (1)
<223> Asp can be Asp or Gln

<220>
<221> MOD_RES
<222> (3)
<223> Ile can be Ile or Val

<220>
<221> MOD_RES
<222> (7)
<223> Gln can be Gln or Arg

<220>
<221> MOD_RES
<222> (9)
<223> Pro can be Pro or Gln

<400> 33
Asp Gly Ile Cys Arg Asn Gln Arg Pro
1 5

<210> 34
<211> 4
<212> PRT
<213> Aplysia

<400> 34
Phe Ala Asp Ser
1

<210> 35
<211> 8
<212> PRT
<213> Aplysia

<220>
<221> MOD_RES
<222> (5)
<223> Ile can be Ile or Leu

<400> 35
Gly Pro Asp Gly Ile Val Ala Asp
1 5

<210> 36
<211> 7
<212> PRT
<213> Aplysia

<220>
<221> MOD_RES
<222> (6)
<223> Lys can be Lys or Gln

<220>
<221> MOD_RES
<222> (7)
<223> Ile can be Ile or Leu

<400> 36
Pro Gly Glu Val Ser Lys Ile
1 5

<210> 37
<211> 15
<212> PRT
<213> Aplysia

<400> 37
Ala Thr Gln Ala Tyr Ala Ala Val Arg Pro Ile Pro Ala Ser Lys
1 5 10 15

<210> 38
<211> 13
<212> PRT
<213> Aplysia

<400> 38
Asp Ser Gly Leu Asp Ile Ala Val Glu Tyr Ser Asp Arg
1 5 10

<210> 39
<211> 12
<212> PRT
<213> Aplysia

<400> 39
Gly Asp Val Pro Tyr Asp Leu Ser Pro Glu Glu Lys
1 5 10

<210> 40
<211> 442
<212> DNA
<213> Aplysia

<400> 40
caagacgggg aagacaagga gtttgacgga gaaatcgta gcgtcagagt gctgaaggcg 60
ttcggcaagc ctggctacgg ttacaagcag ccctcgtgca aggaaggcaa ggactacgtg 120
agcagcggca gcgttcttca cgtgctgcag tgtgccggct tcttcgaggt gtgctacgag 180
gagaggatca ccacccagcc agccacgact gtcgctgcag cagaggtaca atgcaaaaag 240
ttcatcgcaa cccacaaatt ggaggagact gttgatggaa ggatcgtcag catcgagctt 300
gtccagagac tgaagaaaca atccggatac ggtccaagtg gcggttctgg ttatggcaac 360
ggtcatggtc aaagacccgg ttacggatac ggttctggta gtggaagtgg ctacgcccc 420
agaggaggat acaacccaaa ag 442

<210> 41
<211> 147
<212> PRT
<213> Aplysia

<400> 41
Gln Asp Gly Glu Asp Lys Glu Phe Asp Gly Glu Ile Val Ser Val Arg
1 5 10 15

Val Leu Lys Ala Phe Gly Lys Pro Gly Tyr Gly Tyr Lys Gln Pro Ser
 20 25 30
 Cys Lys Glu Gly Lys Asp Tyr Val Ser Ser Gly Ser Val Leu His Val
 35 40 45
 Leu Gln Cys Ala Gly Phe Phe Glu Val Cys Tyr Glu Glu Arg Ile Thr
 50 55 60
 Thr Gln Pro Ala Thr Thr Val Ala Ala Ala Glu Val Gln Cys Lys Lys
 65 70 75 80
 Phe Ile Ala Thr His Lys Leu Glu Glu Thr Val Asp Gly Arg Ile Val
 85 90 95
 Ser Ile Glu Leu Val Gln Arg Leu Lys Lys Gln Ser Gly Tyr Gly Pro
 100 105 110
 Ser Gly Gly Ser Gly Tyr Gly Asn Gly His Gly Gln Arg Pro Gly Tyr
 115 120 125
 Gly Tyr Gly Ser Gly Ser Gly Ser Gly Tyr Ala Pro Arg Gly Gly Tyr
 130 135 140
 Asn Pro Lys
 145

<210> 42
 <211> 462
 <212> DNA
 <213> Aplysia

<400> 42
 taccgcccc gccaccactn tngcaccagc agaaccaacc tgcgagaagc tgtccgtntg 60
 gttcaacgtg ganaagaaat tcgaagggtc cagaatcgtg agtttcaagc tcatccgcct 120
 gttcaacagg tncaagaagt gcaagaaagn ccagtattcc gtgtctggcg atgatgagga 180
 cncattcggt gtcagtggtt gttctggcgt gttccaggtn tgctacgaag aacaaacggc 240
 gcccgtctaca accnccacag aagccccgaa gccagagcca agaagaccca agaggaaaaa 300
 tttcccaatc aaatttngta aacactgatg ggtaaantng acgaccagtg cgtctgcgaa 360
 agaatcatgt tatggttcat gatgtcatgc tcttaataata ggttgtaacg ttttaacgca 420
 tacagacatt aaaactcatt gttcaaaaaa aaaaaaaaaa aa 462

<210> 43
 <211> 155
 <212> PRT
 <213> Aplysia

<220>
 <221> MOD_RES
 <222> (1)..(155)
 <223> Xaa = unknown amino acid or STOP-codon

<400> 43
 Tyr Arg Pro Arg His His Xaa Xaa Thr Ser Arg Thr Asn Leu Arg Glu
 1 5 10 15
 Ala Val Arg Xaa Val Gln Arg Gly Xaa Glu Ile Arg Arg Phe Gln Asn
 20 25 30

Arg Glu Phe Gln Ala His Pro Pro Val Gln Gln Xaa Gln Glu Val Gln
35 40 45

Glu Xaa Pro Val Phe Arg Val Trp Arg Xaa Xaa Gly Xaa Ile Arg Cys
50 55 60

Gln Trp Leu Phe Trp Arg Val Pro Gly Xaa Leu Arg Arg Thr Asn Gly
65 70 75 80

~~Ala Arg Tyr Asn Xaa His Arg Ser Pro Glu Ala Arg Ala Lys Lys Thr~~
85 90 95

Gln Glu Glu Lys Phe Pro Asn Gln Ile Xaa Xaa Thr Leu Met Gly Xaa
100 105 110

Xaa Asp Asp Gln Cys Val Cys Glu Arg Ile Met Leu Trp Phe Met Met
115 120 125

Ser Cys Ser Xaa Xaa Tyr Arg Leu Xaa Arg Leu Thr Arg Tyr Arg His
130 135 140

Xaa Asn Ser Leu Phe Lys Lys Lys Lys Lys Lys
145 150 155

<210> 44
<211> 153
<212> PRT
<213> Aplysia

<220>
<221> MOD_RES
<222> (1)..(153)
<223> Xaa = unknown amino acid or STOP-codon

<400> 44
Thr Ala Pro Ala Thr Thr Xaa Ala Pro Ala Glu Pro Thr Cys Glu Lys
1 5 10 15

Leu Ser Xaa Trp Phe Asn Val Xaa Lys Lys Phe Glu Gly Ser Arg Ile
20 25 30

Val Ser Phe Lys Leu Ile Arg Leu Phe Asn Arg Xaa Lys Lys Cys Lys
35 40 45

Lys Xaa Gln Tyr Ser Val Ser Gly Asp Asp Glu Asp Xaa Phe Val Val
50 55 60

Ser Gly Cys Ser Gly Val Phe Gln Xaa Cys Tyr Glu Glu Gln Thr Ala
65 70 75 80

Pro Ala Thr Thr Xaa Thr Glu Ala Pro Lys Pro Glu Pro Arg Arg Pro
85 90 95

Lys Arg Lys Asn Phe Pro Ile Lys Phe Xaa Lys His Xaa Trp Val Asn
100 105 110

Xaa Thr Thr Ser Ala Ser Ala Lys Glu Ser Cys Tyr Gly Ser Xaa Cys
115 120 125

His Ala Leu Asn Ile Gly Cys Asn Val Xaa Arg Asp Thr Asp Ile Lys
 130 135 140

Thr His Cys Ser Lys Lys Lys Lys Lys
 145 150

<210> 45
 <211> 153
 <212> PRT
 <213> Aplysia

<220>
 <221> MOD_RES
 <222> (1)..(153)
 <223> Xaa = unknown amino acid or STOP-codon

<400> 45
 Pro Pro Pro Pro Pro Xaa Xaa His Gln Gln Asn Gln Pro Ala Arg Ser
 1 5 10 15

Cys Pro Xaa Gly Ser Thr Trp Xaa Arg Asn Ser Lys Val Pro Glu Ser
 20 25 30

Xaa Val Ser Ser Ser Ser Ala Cys Ser Thr Gly Xaa Arg Ser Ala Arg
 35 40 45

Lys Xaa Ser Ile Pro Cys Leu Ala Met Met Arg Xaa His Ser Leu Ser
 50 55 60

Val Val Val Leu Ala Cys Ser Arg Xaa Ala Thr Lys Asn Lys Arg Arg
 65 70 75 80

Pro Leu Gln Xaa Pro Gln Lys Pro Arg Ser Gln Ser Gln Glu Asp Pro
 85 90 95

Arg Gly Lys Ile Ser Gln Ser Asn Xaa Val Asn Thr Asp Gly Leu Xaa
 100 105 110

Xaa Arg Pro Val Arg Leu Arg Lys Asn His Val Met Val His Asp Val
 115 120 125

Met Leu Leu Ile Xaa Val Val Thr Phe Asn Ala Ile Gln Thr Leu Lys
 130 135 140

Leu Ile Val Gln Lys Lys Lys Lys Lys
 145 150

<210> 46
 <211> 9
 <212> PRT
 <213> Aplysia

<220>
 <221> MOD_RES
 <222> (3)
 <223> Ile can be Ile or Val

<400> 46
Asp Gly Ile Cys Arg Asn Arg Arg Gln
1 5

<210> 47
<211> 14
<212> PRT
<213> Aplysia

<400> 47
Asp Ser Gly Leu Asp Ile Ala Val Phe Glu Tyr Ser Asp Arg
1 5 10

<210> 48
<211> 7
<212> PRT
<213> Aplysia

<400> 48
Val Phe Glu Tyr Ser Asp Arg
1 5

<210> 49
<211> 16
<212> PRT
<213> Aplysia

<220>
<221> MOD_RES
<222> (3)
<223> Xaa = any amino acid, in particular Thr

<400> 49
Leu Phe Xaa Tyr Gln Leu Pro Asn Thr Pro Asp Val Asn Leu Glu Ile
1 5 10 15

<210> 50
<211> 10
<212> PRT
<213> Aplysia

<400> 50
Val Ile Ser Glu Leu Gly Leu Thr Pro Lys
1 5 10

<210> 51
<211> 11
<212> PRT
<213> Aplysia

<220>
<221> MOD_RES
<222> (5)
<223> Xaa = any amino acid, in particular Met

<400> 51
Val Ile Leu Ala Xaa Pro Val Tyr Ala Leu Asn
1 5 10

<210> 52
<211> 8
<212> PRT
<213> Aplysia

<400> 52
Val Phe Met Thr Phe Asp Gln Pro
1 5

<210> 53
<211> 10
<212> PRT
<213> Aplysia

<220>
<221> MOD_RES
<222> (6)
<223> Phe can be Phe or Ser

<400> 53
Ser Asp Ala Leu Phe Phe Gln Met Tyr Asp
1 5 10

<210> 54
<211> 18
<212> PRT
<213> Aplysia

<400> 54
Ser Glu Ala Ser Gly Asp Tyr Ile Leu Ile Ala Ser Tyr Ala Asp Gly
1 5 10 15

Leu Lys

<210> 55
<211> 21
<212> PRT
<213> Aplysia

<220>
<221> MOD_RES
<222> (12)
<223> Gln can be Gln or Gly

<400> 55

Asn Gln Gly Glu Asp Ile Pro Gly Ser Asp Pro Gln Tyr Asn Gln Val
1 5 10 15

Thr Glu Pro Leu Lys
20

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

☒ **BLACK BORDERS**

☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**

☒ **FADED TEXT OR DRAWING**

☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**

☐ **SKEWED/SLANTED IMAGES**

☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**

☐ **GRAY SCALE DOCUMENTS**

☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**

☒ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**

☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.